LC AND LC/MS

Your Essential Resource for Columns & Supplies

The Measure of Confidence

Agilent Technologies

LC and LC/MS Columns

The largest portfolio of Fast LC columns, and a broad family of phases across all particle sizes for exceptional flexibility and scalability

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.

- **Poroshell 120 columns** – high efficiency and high resolution with up to 50% less pressure than sub-2 µm columns.
- **ZORBAX Rapid Resolution High Definition (RRHD) columns** – 1.8 µm columns feature improved packing processes to achieve stability up to 1200 bar for use with the Agilent 1290 Infinity LC and other UHPLC instruments and are available in more than 14 phases, plus HILIC.
- **ZORBAX Eclipse Plus columns** – C18 and C8 columns deliver superior peak shape, while the phenyl-hexyl bonded phase and C18 bonded phase for PAH separations expand selectivity options for more applications. All Eclipse Plus phases are available in Fast LC/UHPLC RRHD and RRHT columns, 1.8 µm. For scalability, the Eclipse Plus C18 phase is very similar to the Poroshell 120 EC-C18 phase.
- In addition to Poroshell 120 and RRHD columns, **ZORBAX Rapid Resolution High Throughput (RRHT) columns** are a third Fast LC option with over 140 1.8 µm columns choices. RRHT columns are available in 2.1, 3.0 and 4.6 mm ids, all with 600 bar stability.

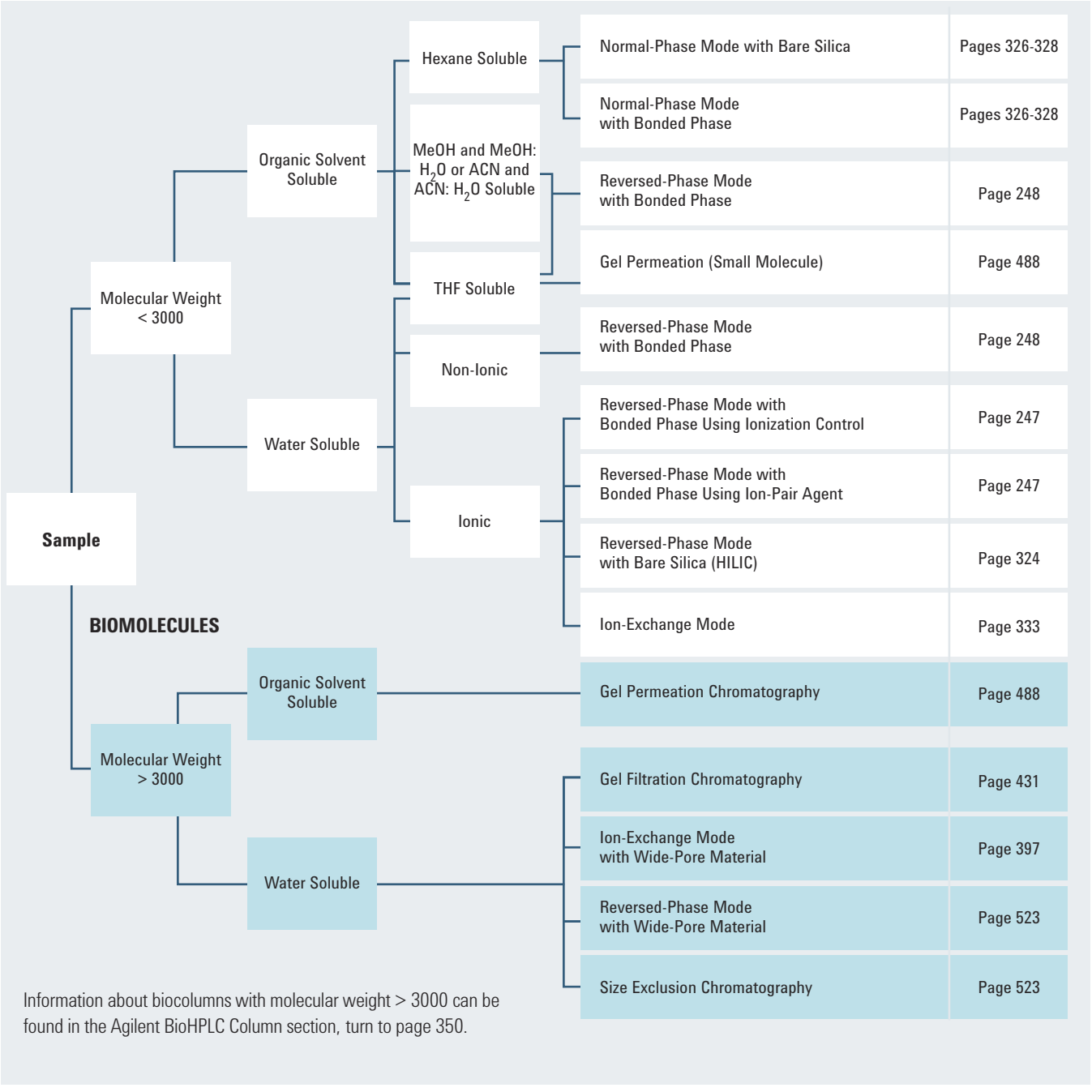
And remember, when you choose Agilent ZORBAX LC columns, you get more than just a dependable product. You also get over 40 years of expertise – along with unmatched technical support – from the world's largest chromatography supplier. On the web, by phone or in person, Agilent helps you solve the problems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

Table of Contents

LC and LC/MS Columns for Small Molecule Separations

HPLC 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.



Information about biocolumns with molecular weight > 3000 can be found in the Agilent BioHPLC Column section, turn to page 350.

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

Quick Guide to Agilent Reversed-Phase Bonded Phases		
ZORBAX RP-HPLC Columns	**Recommended Uses and Applications**	**Page No.**
Poroshell 120	• Superficially porous particles for high efficiency at low pressure • Sub-2 µm efficiency with a 2.7 µm particle • Endcapped and non-endcapped C18 and C8 phases, and a variety of other phases, for selectivity optimization • Compatible with 400 bar and 600 bar LC's	228
Eclipse Plus Available in RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• 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	248
Eclipse XDB Available in RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• Four selectivity choices for flexible method development • High performance over a wide pH range (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	256
StableBond (SB) Available in RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• Basic, acidic, neutral compounds • Exceptional stability at low pH (1-2) • 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	264

(Continued)

Information about biocolumns can be found in the section beginning on page 350

Quick Guide to Agilent Reversed-Phase Bonded Phases

ZORBAX RP-HPLC Columns	Recommended Uses and Applications	Page No.
ZORBAX Rx Available in RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• 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	272
Bonus-RP Available in Fast LC/UHPLC RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• 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	278
Extend-C18 Available in Fast LC/UHPLC RRHD (1200 bar) and RRHT (600 bar) configurations, 1.8 µm	• 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	274
Original ZORBAX Columns	**Recommended Uses and Applications**	**Page No.**
ZORBAX	• 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 • Available in ODS, C8, CN and ODS "Classic" (non-endcapped)	283

TIPS & TOOLS

The LC Handbook: Guide to LC Columns and Method Development

This handy guide makes it easy to choose the right LC column, and contains plenty of tips and tricks to make your job easier and more productive (publication # 5990-7595EN).

Request a copy or download a mobile copy at www.agilent.com/chem/lchandbook

Quick Guide to Additional Agilent Reversed-Phase Columns

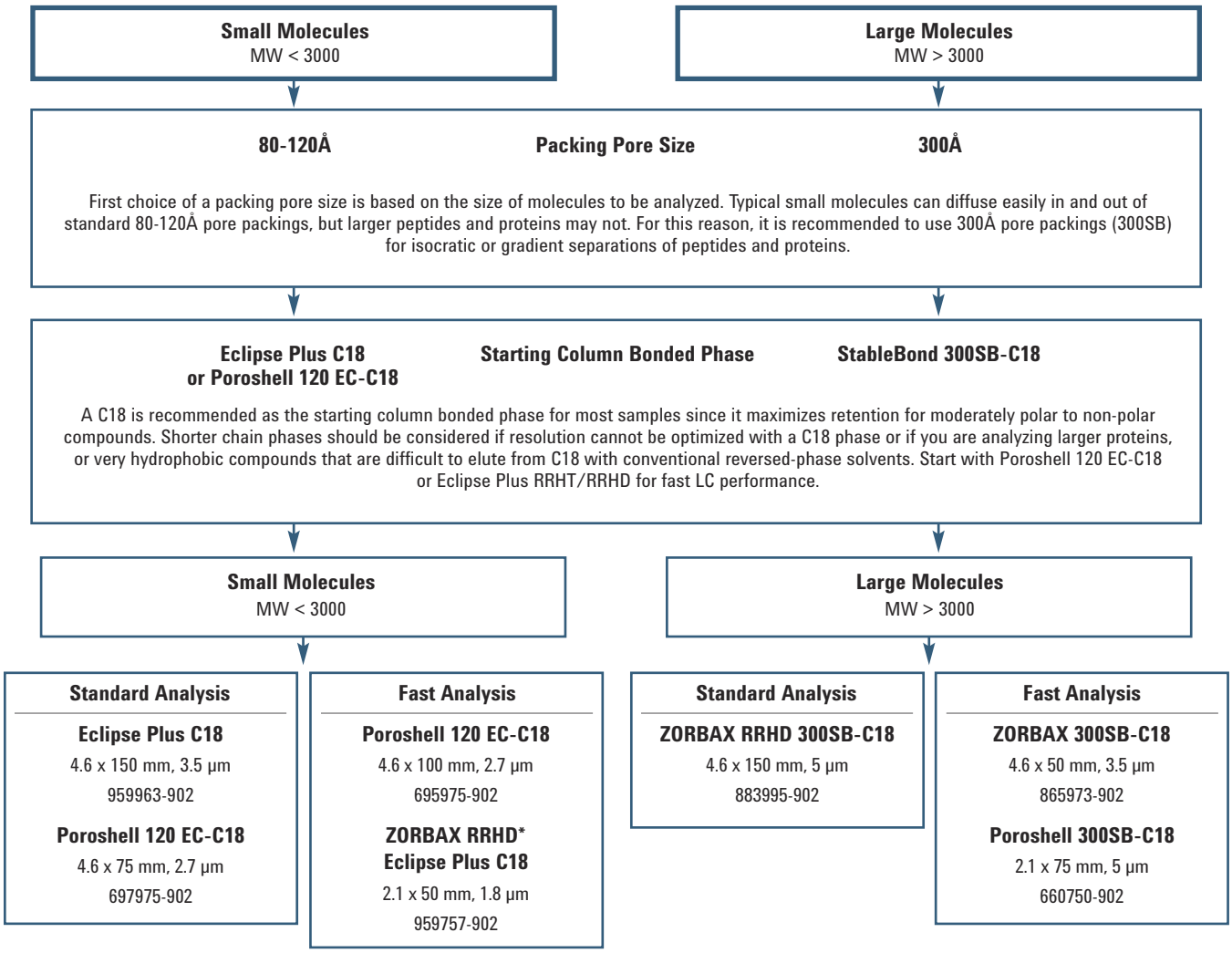
Pursuit Family	Recommended Uses and Applications	Page No.
Pursuit HPLC	• Full range of phases, including C18 and C8 • Diphenyl utilizes strong dipole-dipole hydrogen bonding and pi-pi mechanisms for different selectivity with aromatic compounds • PFP provides excellent separation of polar (halogenated) analytes and positional isomers under standard reversed-phase conditions	287
Pursuit XRs and Pursuit XRs Ultra	• Offer larger surface area and smaller pore size, in complementary phases to Pursuit family • Ultra offers stability to 600 bar, due to special hardware and loading	287
Polaris Family	**Recommended Uses and Applications**	**Page No.**
C18-A and C8-A Available in 3.0, 5.0, and 10 µm (C18-A only)	• C18-A and C8-A offer alternate selectivities for general polar applications • Designed with hydrogen-bond-accepting endcapping	298
Amide-C18 Available in 3.0 and 5.0 µm	• Subtle alternative selectivity due to the absence of steric protection • Utilize an embedded amide, similar to ZORBAX Bonus-RP	298
C18-Ether and C8-Ether Available in 3.0 and 5.0 µm	• Endcapped with an ether group to create a more polar surface for selectivity variation	298
Other Agilent Columns	**Recommended Uses and Applications**	**Page No.**
TC-C18(2) Available in 5 µm	• An excellent choice for mixtures of polar and non-polar compounds, including strong basic compounds	304
HC-C18(2) Available in 5 µm	• High-value, highly retentive option • Carbon load of 17% • Superior peak shape for basic compounds	304

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.



* First choice for use on the 1290 Infinity LC or other UHPLC instruments with 1000+ bar pressure limit.

Information about biocolumns can be found in the section beginning on page 350

Column and Mobile Phase Guidelines: Reversed-Phase

HPLC columns consist of two parts: the column chemistry and hardware. For the proper column chemistry, consult the catalog section for each type of bonded phase. For choosing column hardware and particle sizes, consult the section on column sizes and rapid separations, including Agilent ZORBAX Rapid Resolution HT, Solvent Saver, Capillary and PrepHT columns.

ZORBAX Rapid Resolution
High Throughput (RRHT) Columns

Pore Size Selection

Choose a column packing with small pore (60-120Å) if the solute molecular weight is less than about 3000. Otherwise, use column packing with the 300Å pore size.

Particle Size Selection

The typical particle size for HPLC columns is 5 µm with 3.5 µm and smaller, now common in method development. If high-speed analyses or higher resolution analyses are required, packing with 1.8 µm and 2-3 µm particles can be used. Shorter columns with these particles can produce faster high-resolution separations, with the 1.8 µm particle size providing the highest efficiency and 2.7 µm superficially porous providing similar results. With 1.8, 2.7, 3.5 and 5 µm particle sizes to choose from, start with the smallest particle size for your HPLC or UHPLC – 400 bar, 600 bar, or 1200 bar – to achieve the best results.

Column Configuration

Choosing the best column size for method development has changed dramatically in the past few years. Smaller 3.0 mm id or 2.1 mm id columns are now used more than 4.6 mm id to lower solvent use and achieve compatibility with MS detectors. And shorter 50, 75 and 100 mm long columns can be a great starting choice, with longer columns used only when more resolution is needed or when 3.5 and 5 µm particle sizes are used.

TIPS & TOOLS

Need help selecting the right LC column for your method?
Try the Navigator: A selection tool for LC columns and sample prep.
Look for it online and via your mobile device at **http://navigator.chem.agilent.com**

Silica, Polymers, and Bonded Phase

Base Material

The base material for an LC column is most often high purity silica material with totally porous particles such as that used in most Agilent columns, including ZORBAX, Pursuit, and Polaris. However, more choices are available, including polymer material with high pH stability used in PLRP-S columns and superficially porous silica particles such as those used in Poroshell 120 columns. The high purity Type B silicas, including the ZORBAX Rx-SIl used in ZORBAX Eclipse Plus, and superficially porous Poroshell 120, are an excellent first choice for most methods. Type A silicas, such as ZORBAX SIL, used in Original ZORBAX columns, are still manufactured and used in many methods.

Bonded Phase

A good first choice for bonded phase is C18 or C8, and the recommended starting column choices are Eclipse Plus C18 or Poroshell 120 EC-C18. These two choices provide excellent peak shape and can be used over the pH range 2-9, accommodating most typical LC and LC/MS mobile phases. If the sample solutes of interest are not adequately separated on these columns, CN and Phenyl columns – including Phenyl, Phenyl-Hexyl and Diphenyl – may offer significant differences in selectivity from 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, C8) and peptides and small molecules are separated on longer-chain columns (C18). However, there are many cases 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.

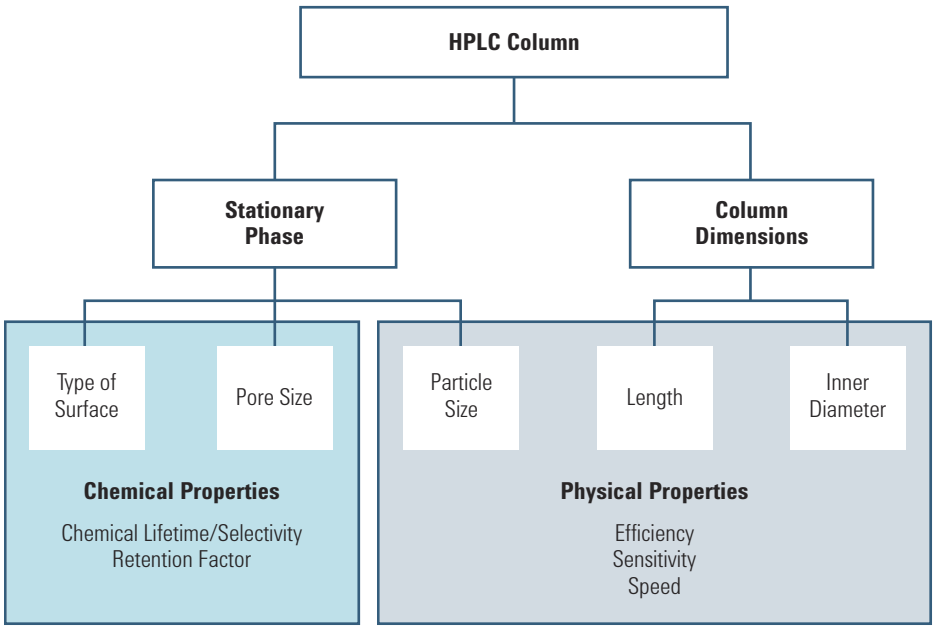
Polymers

When a column is needed that can operate at very low and very high pH, polymeric packings provide an alternative to silica-based materials. Polymeric particles are good for small-scale chromatography, particularly LC/MS, as they are chemically stable and do not leach soluble or particulate species. Reversed-phase spherical polymeric packings used in Agilent PLRP-S columns, for example, are based on a styrene/divinylbenzene copolymer with an inherently hydrophobic surface. No bonded phase is required for reversed-phase chromatography with polymeric particles. These rigid macroporous particles can be coated and derivatized to give a range of functionalities, including weak and strong cation and anion exchangers.

pH and Mobile Phase

The choice of mobile phase for a reversed-phase system starts with selecting the organic modifier. Acetonitrile is the most commonly used organic modifier. However, 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 resolution. A pH 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.



Working with LC/MS

When choosing HPLC columns for LC/MS, chromatographers often need to consider several aspects of their method and separation, typically including resolution, flow rate, and stationary phase choice. Often, for relatively simple analytes, shorter high resolution columns are the best choice. These columns allow for high throughput while maintaining high separation efficiency. Narrow bore Rapid Resolution High Definition (RRHD) for separations (> 600 bar) and Poroshell 120 columns (< 600 bar) offer high resolution even in shorter columns dimensions. For more difficult samples, users should seek longer column lengths.

Since many LC/MS analyses are run at lower flow rates (typically from µL/min flow rates up to 1 mL/min), moving to smaller internal diameter columns is the best choice for the user. Agilent's Solvent Saver (3.0 mm id) and narrow bore (2.1 mm id) will often result in lower solvent usage for the method, and are excellent options for high resolution and higher sensitivity than the larger id columns.

Most often, the best bonded phase choice is an endcapped C18 phase. Eclipse Plus C18 is a high performance endcapped C18 phase available in sub-2 µm RRHD and RRHT column formats. For fast high-throughput separations with LC/MS, Poroshell 120 EC-C18 is an excellent choice. Poroshell has a larger frit, so it's well suited for dirtier LC/MS samples, such as blood plasma, which may often clog columns with smaller porosity frits.

Both Eclipse Plus C18 and Poroshell 120 EC-C18 phases are stable over a wide pH range and are compatible with the volatile buffers such as acetic and formic acids.

TIPS & TOOLS

LC Flow Rate Calculator App

This FREE Smartphone app lets you quickly adjust your flow rate to accommodate other method changes.

Download at **www.agilent.com/chem/lcapp**

Transferring your method to a high efficiency column

High efficiency columns for UHPLC/Fast LC will help you increase your analytical speed and resolution. Depending on the instrument configuration you are using, you may need to make a few adjustments to get the most from these columns.

Because of their high efficiency, very narrow peaks elute from higher efficiency columns quickly. While modern HPLC instrumentation and data systems are able to capture the benefits of these particles, attention to instrumental configuration is important to get the best results.

Steps to transfer your method:

Check the specifications that came with your instrument – Your instrument may already be configured appropriately for high efficiency columns. If not, then continue.

Optimize the data collection rate for LC and LC/MS (at least 40 Hz detector with fast response time for UV) – Set the detector to the fastest setting, then to the second fastest setting and evaluate if the resolution is different.

Use a semi-micro or micro-flow cell – Smaller volume flow cells such as the semi-micro (6 mm/5 µL) or micro (3 mm/2 µL) are recommended for best performance. There are newer cartridge flow cells (e.g. the Ultra Low-Dispersion Max-Light Ultra Flow Cell, P/N G4212-60007) designed to optimize UHPLC instrument performance.

TIPS & TOOLS

For the Agilent 1290 Infinity LC, in situations requiring extremely low dead volumes, use the ultra-low dispersion kit, which includes an ultra-low dispersion flow cell and 0.08 mm id capillaries.

Minimize tubing volume in the instrument – Use Red (0.12 mm id) tubing instead of Green (0.17 mm id) as it has only half of the volume that the sample has to travel through. This cuts down extra column band broadening. Ensure that your connections are as short as possible. The key locations to check are:

- The autosampler needle seat
- The autosampler to the Thermostatted Column Compartment – or 'TCC'
- The TCC to the column
- The column to the flow cell, including the internal diameter of the integral flow cell inlet capillary

All of these specific capillaries can be ordered individually from Agilent, in the lengths you need, and for your instrument.

Turn to pages 36-39.

Scale your gradient profile and injection volume – If using gradient elution, scale the gradient profile and injection volume to the new smaller column to quickly transfer the method and avoid overloading. For isocratic and gradient elution, make sure that you scale the injection volume to match the overall column volume.

Minimize injection sample dispersion in the column – Use an injection solvent with solvent strength that is equivalent to or weaker than the mobile phase, especially when using an isocratic method. This is good practice in general for any column, and more important with high efficiency columns.

TIPS & TOOLS

See a video that takes you through these steps at
www.agilent.com/chem/poroshell120video

Also, check out the LC Method Translator Tool at
www.agilent.com/chem/lcmethodtranslator

Take care to make proper connections – Agilent recommends Swagelok fittings with front and back ferrules, which give best sealing performance throughout our LC system (use this on the instrument connections, i.e. valves, heaters, etc). Polyketone fittings are highly recommended for up to 600 bar. Use this fitting (P/N 5042-8957) on column connections with Poroshell 120. For RRHD columns, use Agilent's removable 1200 bar fitting (P/N 5067-4733).

Optimize your flow rate – For Poroshell 120, if you're using a 2.1 mm id, the suggested starting flow rate is 0.42 mL/min; for 3.0 mm id Poroshell 120 columns, we suggest starting at 0.85 mL/min, and for 4.6 mm id, we suggest starting at 1.5 - 2 mL/min.

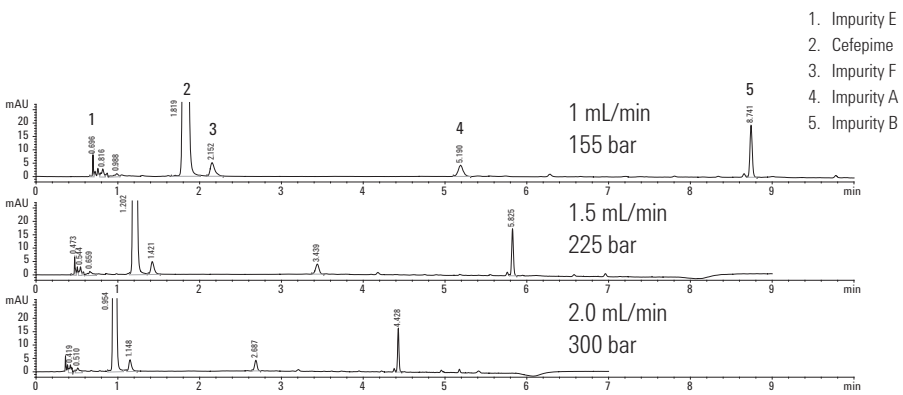
1200 bar removable fitting (SV), 5067-4733

Fast analysis of cefepime and related impurities

Column: **Poroshell 120 EC-C18 697975-902 4.6 x 75 mm, 2.7 µm**

Instrument: Agilent 1200 Infinity Series Rapid Resolution LC System

Detector: DAD, 254 nm



VHP FITTINGS

Agilent's 1200 bar removable fitting (for 1/16 in od capillaries) consists of a stainless steel screw, an internal stainless steel ferrule and a front ferrule in PEEK. The fitting can be used throughout the flow path, but because it can be re-used without losing tightness, it is especially suitable for the connection between the heat exchanger and the column. This new and improved fitting replaces the standard stainless steel Swagelok fitting which was not removable. The Very High Pressure (VHP) fitting is available in three sizes – short (P/N 5067-4733), long (P/N 5067-4738) and extra long (P/N 5067-4739). The short fitting is the one that is most commonly used, and will be appropriate 90% of the time. In some cases, if using columns with longer nuts, a longer fitting will be needed.

Agilent LC Columns Overview: Small Molecules

Start with Poroshell 120 for Fast LC performance on any HPLC – phases align with ZORBAX family.

Poroshell 120

Up to 50% less pressure than sub-2 µm; a total lab productivity enhancer

1.7 µm solid core; 0.5 µm porous outer layer for a 2.7 µm particle, id's: 4.6 mm, 3.0 mm, 2.1 mm, Lengths: 30-150 mm.

New phases coming soon! Check **www.agilent.com/chem/poroshell120**

Compatible with HPLC and UHPLC instruments. Suitable for analysis of acids, bases, and neutrals. Also great for peptide mapping.

Poroshell 120 is for any lab looking for increased analytical speed and resolution with less backpressure.

Poroshell 120 SB-C18 (USP L1), SB-C8
Carbon Load: SB-C18 - 7.5%, SB-C8 - 4.5%

Poroshell 120 EC-C18 (USP L1), EC-C8** (USP L1), Phenyl-Hexyl (USP L11)**
Carbon Load: Phenyl-Hexyl - 8%

Poroshell 120 EC-CN (USP L10)

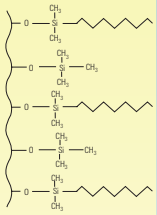
****Best Phase for Method Development**

ZORBAX Family

ZORBAX Eclipse Plus**

RRHD: 1.8 µm, stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 30-250 mm
IDs: 4.6 mm, 3.0 mm, 2.1 mm, 1.0 mm; Prep

C18 (USP L1),
C8 (USP L7),
Phenyl-Hexyl (USP L11),
PAH (USP L1)



High performance and excellent peak shape with acids, bases and neutrals.

Sample Applications
Environmental: EPA Method 1694, Illicit and prescribed drugs in wastewater
Food Safety: Quinolone antibiotics
Pharmaceutical: Chloramphenicol, Simvastatin, Chrysophenol (TCM), amphetamine, ranitidine

Double Endcapped
(except PAH, which is not endcapped)
Temp limit: 60 °C
Pore size: 95Å
Surface area:
160 m^2/g

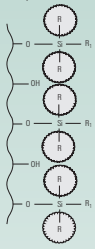
Particle sizes:
1.8, 3.5, 5 µm
pH: 2.0-9.0 for C18, C8; 2.0-8.0 for PAH, Phenyl-Hexyl
Carbon load:
C18: 9%; C8: 7%;
Phenyl-Hexyl: 9% ;
PAH: 14%

Best all around – exceptional peak shape, efficiency, resolution, and lifetime

ZORBAX StableBond

RRHD: 1.8 µm, stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 20-250 mm
IDs: 4.6 mm , 3.0 mm, 2.1 mm, 1.0 mm;
Prep, Capillary (C18)

SB-C18 (USP L1),
SB-C8 (USP L7),
SB-C3 (USP L56),
SB-Phenyl (USP L11),
SB-CN (USP L10),
SB-Aq



High performance with acids, bases, and neutrals with superior lifetime at low pH.

Sample Applications
Chemical/Industrial: Triton
Environmental: Organic acids, pesticides in drinking water
Food Safety: Anthocyanine, parabenes, melamine
Pharmaceutical: Analgesics, anesthetics, traditional Chinese medicine

Non-Endcapped
Temp limit: 80 °C
(90 °C for SB-C18)
Pore size: 80Å
Surface area:
180 m^2/g
Particle sizes:
1.8, 3.5, 5, 7 µm

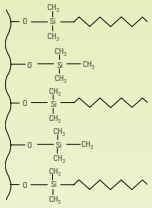
pH: 1.0-8.0
(0.8-8.0 for SB-C18)
Carbon Load:
C18: 10%; C8: 5.5%;
C3: 4%; Phenyl: 5.5%;
CN: 4%,
Aq: Proprietary

Best for low pH mobile phases – great for method development

ZORBAX Eclipse XDB

RRHD: 1.8 µm, stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 15-250 mm
IDs: 4.6 mm, 3.0 mm, 2.1 mm, 1.0 mm;
Capillary and Prep

C18 (USP L1),
C8 (USP L7),
Phenyl (USP L11),
CN (USP L10)



Good peak shape for basic, acidic, and neutral compounds with high performance over a wide pH range (pH 2-9). eXtra Dense Bonding and double endcapping help give this column a long lifetime.

Sample Applications
Environmental: Herbicides/pesticides, steroids in water
Food Safety: Food colors, aromatic flavorings, mycotoxins, epoxyphenolic-based can coatings
Pharmaceutical: Goldenseal and related alkaloids, antidepressants, triamcinolone

Double Endcapped
Temp limit: 60 °C
Pore size: 80Å
Surface area:
180 m^2/g
Particle sizes:
1.8, 3.5, 5, 7 µm

pH: 2.0-9.0
(2.0-8.0 for CN)
Carbon load:
C18: 10%; C8: 7.6%;
Phenyl: 7.2%;
CN: 4.3%

High performance over a wide pH range

Pursuit/ Pursuit XRs

Lengths: 30-250 mm
IDs: 2.0 mm, 3.0 mm, 4.6 mm; Prep

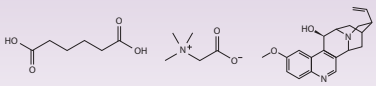
C18 (USP L1), **C8** (USP L7),
Diphenyl (USP L11), **PFP** (USP L43),
PAH (USP L1), **Si** (USP L3)

Pursuit XRs offers higher loadability and Pursuit XRs Ultra is loaded for higher pressure stability.

Endcapped
(except Pursuit XRs Si)
Pore Size: 200Å (Pursuit),
100Å (Pursuit XRs)
Surface area: 200 m^2/g (Pursuit); 440 m^2/g (Pursuit XRs)
Particle Sizes:
3, 5, 10 µm

pH: 2.0-9.0
Carbon Load:
Pursuit C18: 12.9%;
Pursuit C8: 7.4%;
Pursuit Diphenyl: 7.3%;
PFP: 6.3%; XRs C18: 22%; XRs Ultra C18: 23.3%; XRs Ultra C8: 15%; XRs Ultra Diphenyl: 14.6%

Reliable Selectivity Alternatives



Endcapped: EC-C18, EC-C8, Phenyl-Hexyl, Bonus-RP (triple), EC-CN
Non-endcapped: SB-C18, SB-C8 and SB-Aq
Temp Limit: 60 °C (EC-C18, EC-C8, Phenyl-Hexyl, Bonus-RP); 80 °C (SB-C8, SB-Aq); 90 °C (SB-C18)
Pore Size: 120Å ; Surface Area: 130 m^2/g; pH: 2.0-8.0 (EC-C18, EC-C8, Phenyl-Hexyl); 1.0-8.0 (SB-C18, SB-C8, SB-Aq); 2.0-9.0 (Bonus-RP); Carbon Load: 8% (EC-C18); 7% (EC-C8)

Poroshell 120 Bonus-RP (USP L60)
Carbon Load - 7.5%

Poroshell 120 SB-AQ
Carbon Load: Proprietary

POLAR Compounds

ZORBAX Extend-C18

RRHD: 1.8 µm, stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 20-250 mm
IDs: 4.6 mm, 3.0 mm, 2.1 mm, 1.0 mm

C18 (USP L1)

High efficiency and long life at high pH – up to pH 11.5. Improve retention, resolution and peak shape of basic compounds. High sensitivity for LC/MS separations of peptides. Unique bidendate bonding and double endcapping provides high pH stability.

Sample Applications
Environmental: EPA 8330 (explosives)
Food Safety: Aflatoxins, mycotoxins
Pharmaceutical: Antihistamines, xanthines

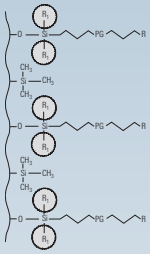
Double-Endcapped
Temp limit: 60 °C
Pore Size: 80Å
Surface area:
180 m^2/g
Particle sizes:
1.8, 3.5, 5 µm
pH: 2.0-11.5
Carbon load: 12.5%

A good option for separations at high pH

ZORBAX Bonus-RP

RRHD: 1.8 µm, stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 30-250 mm
IDs: 4.6 mm, 3.0 mm, 2.1 mm, 1.0 mm; Prep

Bonus-RP (USP-L60)



Polar-embedded to improve peak shapes; for basic compounds at low and mid pH.

Sample Applications
Environmental: Triazine pesticides
Food Safety: Hydroxymethylfurfural
Pharmaceutical: Antifungal medications, anorectics, ulcer medications

Triple-Endcapped
Temp limit: 60 °C
Pore size: 80Å
Surface area:
180 m^2/g
Particle sizes:
1.8, 3.5, 5 µm
pH: 2.0-9.0
Carbon load: 9.5%

Alternative selectivity to alkyl, phenyl, cyano phases

SB-AQ

RRHD: 1.8 µm stable to 1200 bar;
RRHT: 1.8 µm, 600 bar
Lengths: 20 - 250 mm
IDs: 4.6 mm, 3.0 mm, 2.1 mm; Prep

ZORBAX SB-Aq

Proprietary phase ideal for polar compounds and high aqueous conditions.

Sample Applications
Environmental: Pesticides in drinking water
Food Safety: Pesticides in food
Pharmaceutical: Water-soluble vitamins

See ZORBAX StableBond for specification and structure.

Exceptional lifetime at low pH – no endcapping

Polaris

Lengths: 30-250 mm,
(available in
3 µm and 5 µm particles)
IDs: 2.0 mm, 3.0 mm, 4.6 mm; Prep

C18-A (USP L1), **C8-A** (USP L7),
C18-Ether (USP L1), **C8-Ether** (USP L7),
Amide-C18 (USP L60), **NH2** (USP L8),
Si-A (USP L3)

Hydrogen-bond accepting and ether group endcapping provide alternate selectivities.

Sample Applications
Environmental: Triazine pesticides
Food Safety: Hydroxymethylfurfural
Pharmaceutical: Antifungal medications, anorectics, ulcer medications

Endcapped
Pore size: 180Å
Surface Area:
200 m^2/g
Particle Sizes:
3, 5, 10 µm
pH: 2.0-9.0
Carbon load: Polaris C18-A: 13.8%; Polaris C8-A: 7.4%; Polaris C18-Ether: 12.1%; Polaris C8-Ether: 7.1%

More options for Polar Compounds

Information about biocolumns can be found in the section beginning on page 350

Looking for a HILIC column?

HILIC Plus is a HILIC column based on Eclipse Plus silica for excellent peak shapes

Poroshell 120 HILIC: 2.7 µm, stable to 600 bar

Non-bonded silica
Pore size: 95Å (120Å, Poroshell 120)
Surface Area: 160 m^2/g(130 m^2/g for Poroshell 120)
Particle Sizes: 1.8, 2.7, 3.5 µm
pH: 0-8.0

RRHD: 1.8 µm,
stable to 1200 bar
Lengths: 50, 100, 150 mm
IDs: 4.6 mm (3.5 µm only), 3.0 mm, 2.1 mm

High sensitivity for LC/MS applications and recommended for EPA 1694.

Method Development from pH 1-12

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.

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 with 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 five different StableBond bonded phases: SB-C18, SB-C8, SB-Phenyl, SB-CN, and SB-C3. For polar analytes, try Bonus-RP, SB-Aq or the Polaris family, including C18-A, C8-A, C18-Ether and Amide-C18 phases.

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.

TIPS & TOOLS

LC Method Translator

Use this online tool to quickly factor in changes to column length, diameter, flow rate, and more – and to calculate method adjustments. This is particularly useful for gradient methods.

To download, go to **www.agilent.com/chem/lcmethodtranslator**

Choose Agilent ZORBAX Eclipse Plus or Poroshell 120 for method development at mid pH (pH 4-9)

There are some samples that may not be resolved at low pH or may have better solubility and stability at mid pH. The Eclipse Plus C18 and Poroshell 120 EC-C18 columns can be used at the mid pH range for method development. The Eclipse Plus column is stable 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.

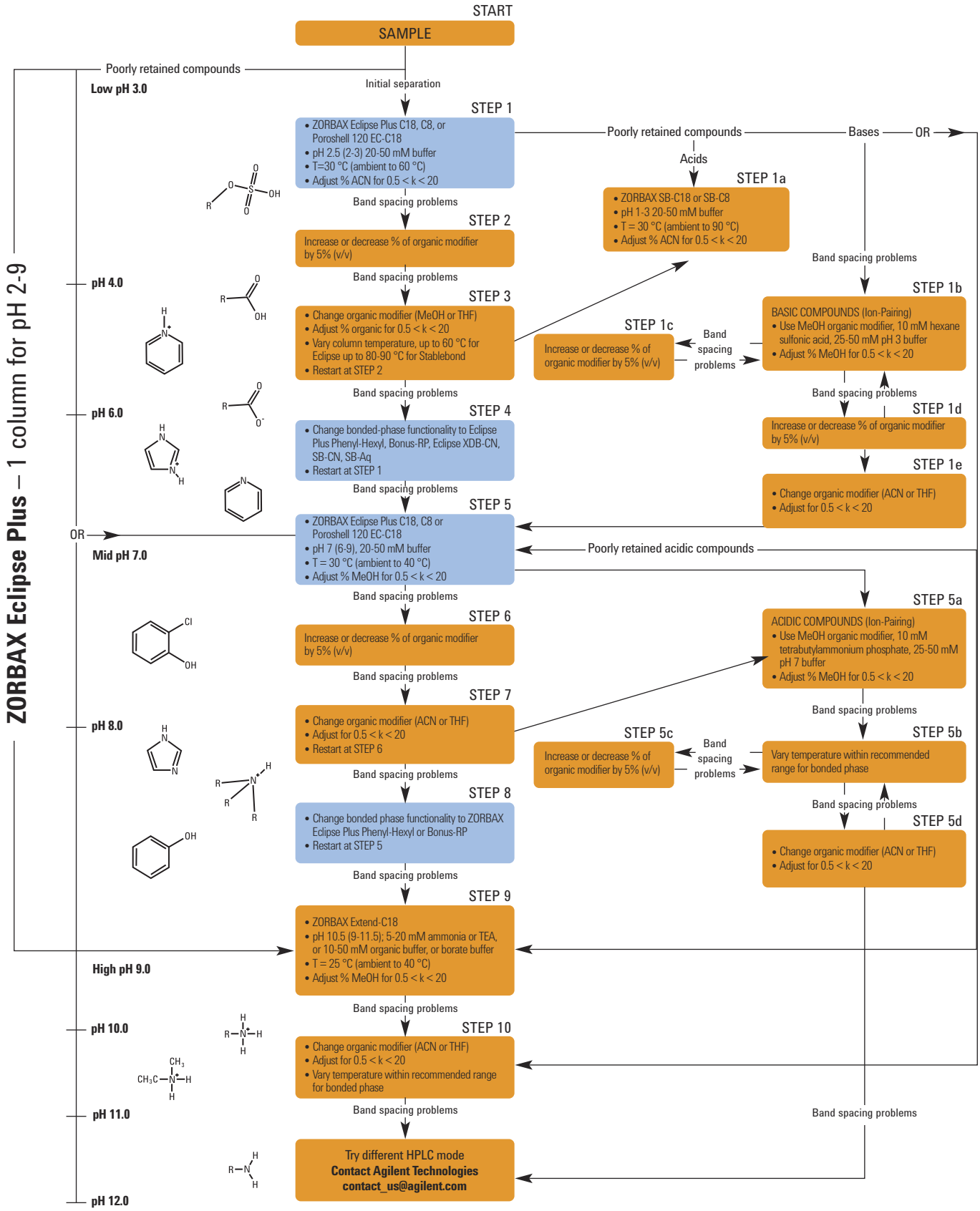
At mid pH, basic compounds (e.g., amines) may still have a positive charge and the silanols on the silica surface may have a negative charge. Therefore covering as many silanols as possible leads to the best peak shape at mid pH. This makes the Eclipse Plus C18 the best starting choice for a column at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

Choose Agilent ZORBAX Extend-C18 columns for method development at high pH (pH 9-12)

At low or mid pH, some separations of basic compounds may still not have enough retention or the desired selectivity. For these samples, high pH separations may be appropriate. Until recently, high pH separations on silica-based columns were avoided because of short column lifetimes, due to dissolution of the underlying silica gel. Special bonded phases such as the ZORBAX Extend-C18, can protect the silica from dissolution, so that a reasonable column lifetime can be achieved and the selectivity advantages of high pH can be explored.

The mobile phase buffer choices at high pH with the Extend-C18 column are organic buffers like triethylamine and ammonium hydroxide. These buffers are best used with methanol as the organic modifier to extend the column lifetime at high pH. This is another good option to consider when working with high pH and PLRP-S columns, which are made from a polymeric material.

Method Development Guidelines from Low to High pH



Guard Columns

The Value of Guard Columns

Guard columns can help extend the life of your analytical column. Choosing to use guard columns can help reduce operating expenses, by reducing the frequency of analytical column replacement.

The guard column prevents damage caused by particulate matter and strongly adsorbed material. To maintain an adequate capacity for sample impurities, choose a guard column with an internal diameter similar to the column internal diameter. Ideally, the packing of the guard column should be the same as the analytical column so that the chromatography of the analytical column is not altered.

Guard columns contribute to the separation, so you should include a guard column in-line during method development.

Agilent UHPLC guards provide protection for high-efficiency Poroshell 120 and ZORBAX RRHD and RRHT columns, without reducing performance. Part numbers for all guard columns are incorporated into the different product family tables.

Judging when to replace a guard column can be difficult. As a rough guide, if plate number, pressure or resolution change by more than 10%, the guard column probably needs replacing. You will need to make a judgment call on how often to replace your guard columns based on your application type. It is always preferable to change the guard column sooner rather than later.

UHPLC Guard, 1200 bar, 821725-903

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: Standalone system	High efficiency, standalone, low-dead-volume cartridge	Seals up to 5000 psi (340 bar) or 3000 psi with a PEEK fitting
		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, Standalone 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: Standalone 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: Standalone 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
PL	Polymeric Analytical Column and Guard Cartridge	High efficiency	Inexpensive
		Low dead volume	Rapid cartridge changes
		Reusable holder	Extends column lifetime
CS	ChromSep Column Hardware: Complete systems and replacement cartridges	Easy, no-dead-volume assembly	Economical format No tools required Modular flexibility
MG	MetaGuard Column Hardware: Complete systems and replacement cartridges	Easy, no-dead-volume assembly	Economical format No tools required Modular flexibility
UG	Agilent Fast Guards for UHPLC	Requires no special hardware – connects right to the analytical column	Extends column lifetime without impacting performance
		Available in matching phases for Poroshell 120, RRHD and RRHT columns	

*Look for these icons to help you select the proper guard cartridges and columns.

Cartridge/Guard Cartridge Systems Compatibility Guide*

Icon	Column Type	Guard Cartridge Holder	ID (mm)	Phases
AC	Cartridge column cartridge holder 5021-1845 	Guard cartridge (internal system) cartridge holder 5021-1845 	2.0 3.0 4.0 4.6	LiChrospher Nucleosil Purospher Superspher ZORBAX
ZGC	Standard fitting 	Column guard cartridge (standalone) cartridge holder 820999-901 	2.1 3.0 4.6	ZORBAX
RR	Rapid Resolution cartridge holder 820555-901 	No guard cartridge holder	4.6	ZORBAX
P	Semi-preparative column 	Semi-prep guard cartridge (standalone) cartridge holder 840140-901 	9.4	ZORBAX

(Continued)

Cartridge/Guard Cartridge Systems Compatibility Guide*

Icon	Column Type	Guard Cartridge Holder	ID (mm)	Phases
PT	PrepHT	Guard cartridge 820444-901 	21.2	ZORBAX Agilent Prep
PL	Analytical 	Guard cartridge holder (PL1310-0016) and PLRP-S guard cartridges, 2/pk (PL1612-1801) 	3.0	PLRP-S
MG	Single replacement column 	No guard cartridge holder	1.0 2.0 4.6	Pursuit Pursuit XRs Polaris phases
UG NEW!	Fast Guards for UHPLC: Single replacement guard column 	No guard cartridge holder	2.1 3.0 4.6	Poroshell 120: EC-C18 EC-C8 SB-C18 Phenyl-Hexyl Sub-2 µm: Eclipse Plus C18 Eclipse XDB-C18 SB-C18 SB-C8

*Standalone guard cartridges fit all cartridge and standard fitting columns available from Agilent. All columns without icons are standard fitting columns.

Fast Columns for Reversed-Phase HPLC/UHPLC

The past decade has seen a steady increase in the efficiency and speed of chromatography, starting with smaller particle sizes, that enable higher resolution, and continuing with new technological advances in particle design – superficially porous particles – that enable these same resolution enhancements with lower backpressure.

Designed especially for high-productivity analysis (Fast LC), Agilent ZORBAX and Poroshell columns are the best first choice for any analysis, because they give you:

- The productivity you need to stay ahead of your competition: technological advances like sub-2 µm particles and superficially porous Poroshell 120 columns deliver increased speed and resolution.
- Flexibility and method scalability from lab to lab and around the world – for small molecule and biomolecule analyses.
- Unbeatable chromatographic performance: ZORBAX silica – the base silica used for all ZORBAX and Poroshell 120 columns – is ultra-pure, very strong, and highly uniform for ultimate reliability.
- The broadest range of phases and column configurations to suit your specific application needs.

Recommendations for Fast LC Columns

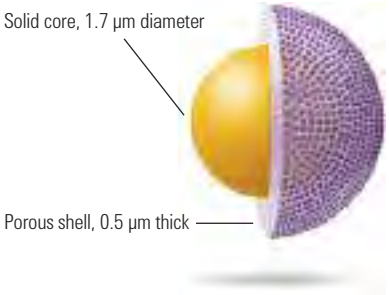
Your Lab Situation	Agilent Recommends	Rationale
You're using both UHPLC (1000+ bar) and HPLC instruments (e.g. Agilent 1290 Infinity LC and 1260 Infinity LC – 600 bar)	1. Poroshell 120 2. ZORBAX RRHD 1.8 µm	Poroshell 120 is an easy column to use on both instrument types. ZORBAX RRHD will help you optimize the capabilities of the 1290 Infinity LC for UHPLC.
Only 400-600 bar HPLCs – Agilent 1200s, Agilent 1100s (400 bar) as well as the 1220 Infinity LC or 1260 Infinity LC (600 bar)	1. Poroshell 120 2. ZORBAX Eclipse Plus 3.5 µm and 5 µm	With Poroshell 120, you can enhance the performance of older 400-bar instruments, and also get even better performance from newer 600 bar UHPLC instruments. For established methods that you can't transfer, the ZORBAX Eclipse Plus column will provide exceptional peak shape and performance.
A mix of UHPLC instruments (Agilent 1290 Infinity LC, other 1000+ bar instruments) and some HPLC instruments (e.g. 1200 LC)	1. ZORBAX RRHD 1.8 µm 2. Poroshell 120	ZORBAX RRHD can deliver optimum performance on all these instruments. Poroshell 120 can be used on the 600 bar instruments to optimize their performance.

TIPS & TOOLS

Agilent CrossLab offers a range of PEEK capillaries and tubing. Used in combination with the right fittings, they provide an inert surface for the Fast LC of sensitive biomolecules. Turn to page 130.

Poroshell 120

- High efficiency and high resolution, with up to 50% less backpressure than sub-2 µm columns
- 2 µm frit, for rugged performance with dirty samples
- Compatible with 400 bar and 600 bar LCs, as well as UHPLC instruments
- An expanding family of bonded phases to align with the ZORBAX Family, for reliable scalability
- Excellent selectivity and peak shapes
- Designed for exceptional reproducibility



Agilent Poroshell 120 columns are a 2.7 µm particle with a 1.7 µm solid core and 0.5 µm porous outer layer. This small particle size provides high efficiency, similar to sub-2 µm columns, but with 40-50% less pressure. These high efficiency, high resolution columns can be used on any type of LC. The porous outer layer and solid core limit diffusion distance and improve separation speed while the narrow particle size distribution improves efficiency and resolution. The columns can support high pressure and multiple columns can be used for the highest resolution and efficiency possible. The same principles are used in Poroshell 300 columns, ideal for fast, high resolution separations of biomolecules.

Column Specifications

Bonded Phase	Pore Size	Temp Limits	pH Range	Endcapped	Carbon Load	Surface Area
EC-C18	120Å	60 °C	2.0-8.0	Double	10%	130 m^2/g
EC-C8	120Å	60 °C	2.0-8.0	Double	5%	130 m^2/g
Phenyl-Hexyl	120Å	60 °C	2.0-8.0	Double	9%	130 m^2/g
SB-C18	120Å	90 °C	1.0-8.0	No	8%	130 m^2/g
SB-C8	120Å	80 °C	1.0-8.0	No	5.5%	130 m^2/g
SB-Aq	120Å	80 °C	1.0-8.0	No	Proprietary	130 m^2/g
Bonus-RP	120Å	60 °C	2.0-9.0	Triple	9.5%	130 m^2/g
EC-CN	120Å	60 °C	2.0-8.0	Double	3.5%	130 m^2/g
HILIC	120Å	60 °C	0.0-8.0	No	N/A	130 m^2/g

Specifications represent typical values only

TIPS & TOOLS

Watch the Poroshell 120 Method Transfer Video to learn how easy it is to transfer existing methods to Poroshell 120 at **www.agilent.com/chem/poroshell120video**

Poroshell 120
(Maximum pressure: 600 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	EC-C18 USP L1	EC-C8 USP L7	Phenyl-Hexyl USP L11	SB-C18 USP L1	SB-C8 USP L7	SB-Aq	Bonus-RP USP L60
	Analytical	4.6 x 150	2.7	693975-902	693975-906	693975-912	683975-902	683975-906	683975-914	693968-901
	Analytical	4.6 x 100	2.7	695975-902	695975-906	695975-912	685975-902	685975-906	685975-914	695968-901
	Analytical	4.6 x 75	2.7	697975-902	697975-906		687975-902			
	Analytical	4.6 x 50	2.7	699975-902	699975-906	699975-912	689975-902	689975-906	689975-914	699968-901
	Analytical	4.6 x 30	2.7	691975-902	691975-906		681975-902			
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	2.7	820750-911	820750-913	820750-914	820750-912			
	Solvent Saver	3.0 x 150	2.7	693975-302	693975-306	693975-312	683975-302	683975-306	683975-314	693968-301
	Solvent Saver	3.0 x 100	2.7	695975-302	695975-306	695975-312	685975-302	685975-306	685975-314	695968-301
	Solvent Saver	3.0 x 75	2.7	697975-302	697975-306		687975-302			
	Solvent Saver	3.0 x 50	2.7	699975-302	699975-306	699975-312	689975-302	689975-306	689975-314	699968-301
	Solvent Saver	3.0 x 30	2.7	691975-302	691975-306		681975-302			
UG	UHPLC Guard, 600 bar, 3/pk	3.0 x 5	2.7	823750-911	823750-913	823750-914	823750-912			
	Narrow Bore	2.1 x 150	2.7	693775-902	693775-906	693775-912	683775-902	683775-906	683775-914	693768-901
	Narrow Bore	2.1 x 100	2.7	695775-902	695775-906	695775-912	685775-902	685775-906	685775-914	695768-901
	Narrow Bore	2.1 x 75	2.7	697775-902	697775-906		687775-902			
	Narrow Bore	2.1 x 50	2.7	699775-902	699775-906	699775-912	689775-902	689775-906	689775-914	699768-901
	Narrow Bore	2.1 x 30	2.7	691775-902	691775-906		681775-902			
UG	UHPLC Guard, 600 bar, 3/pk	2.1 x 5	2.7	821725-911	821725-913	821725-914	821725-912			

Poroshell 120 Columns

Environmental phenols on Poroshell 120

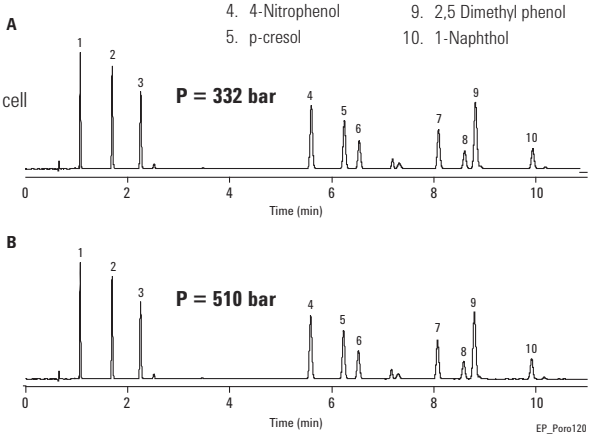
Column A: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Column B: **Eclipse Plus C18**
959964-902
4.6 x 100 mm, 1.8 µm

Gradient: A: Water 0.1% formic acid
B: Acetonitrile 0.1% formic acid
2 mL/min
Initial: 8% B
10 min: 30% B

Detector: 275 nm, 2 mm flow cell
Injection: 10 µL
Agilent 1200 SL 40 °C
No pulse damper
No mixer 3 µL heater

1. Hydroquinone
2. Resorcinol
3. Catechol
4. 4-Nitrophenol
5. p-cresol
6. o-cresol
7. 2-Nitrophenol
8. 2,3 Dimethyl phenol
9. 2,5 Dimethyl phenol
10. 1-Naphthol



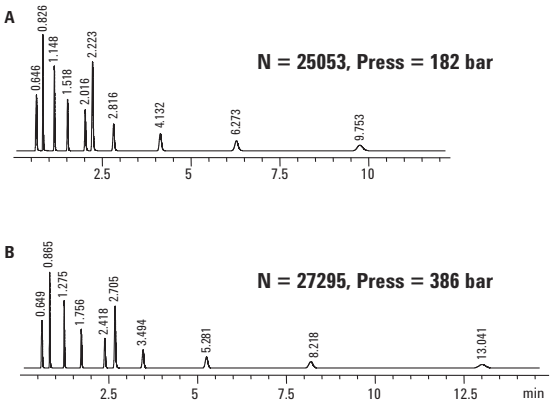
Poroshell 120 provides sub-2 µm like efficiency at lower pressure.

UHPLC efficiency at HPLC pressures

Column A: **Poroshell 120 EC-C18**
695975-302
3.0 x 100 mm, 2.7 µm

Column B: **Eclipse Plus C18**
959964-302
3.0 x 100 mm, 1.8 µm

Mobile Phase: 60% Acetonitrile:40% water
Flow Rate: 0.58 mL/min
Temperature: 26 °C
Injection Volume: 4 µL
Detector: DAD Sig = 254,4 nm
Ref = 360,100 nm
Sample: RRLC checkout sample (P/N 5188-6529) spiked w/50 µL 2 mg/mL thiourea in water/acetonitrile (65:35)



For this sample of neutral alkylphenones, the Poroshell 120 column delivered >90% of the efficiency attained by the 1.8 µm column. Also note that the pressure on the Poroshell 120 column is about 50% of the pressure on the 1.8 µm column.

HPLC separation of 12 phenols performed in just 5 minutes – and under 400 bar – using an Agilent Poroshell 120 EC-C18 column

Column: **Poroshell 120 EC-C18**
699975-902
4.6 x 50 mm, 2.7 µm

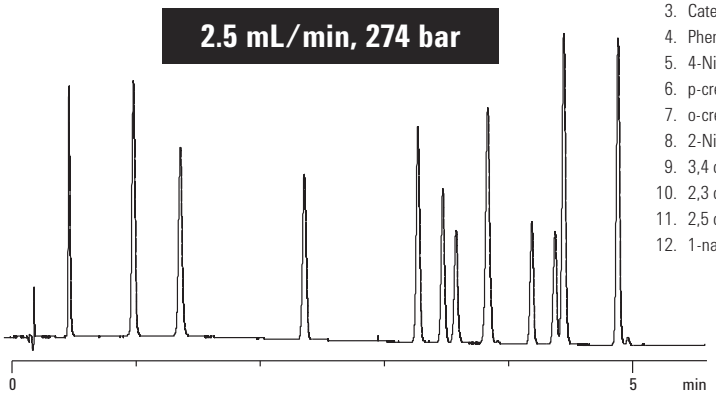
Mobile Phase: Solvent A: Water with 0.1% formic acid
Solvent B: Acetonitrile

Gradient: 5% B in 0.8 min
60% B in 6.8 min
1200 SL controlled temperature
at 25 °C 2 mm flow cell

Detector: DAD, 270 nm

Importantly, the flow rate was kept to 2.5 mL/min, reducing the amount of mobile phase consumed per analysis to about 15 mL.

Agilent Poroshell 120 gives high efficiency, high resolution separations quickly at HPLC pressures.



1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol

12 phenols analyzed using a longer (4.6 x 100 mm) Agilent Poroshell 120 EC-C18 column

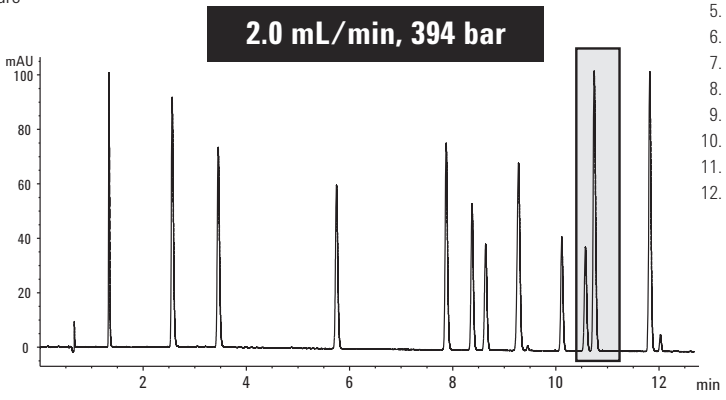
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: Solvent A: Water with 0.1% formic acid
Solvent B: Acetonitrile

Gradient: 5% B in 2 min
60% B in 17 min
1200 RRLC SL controlled temperature
at 25 °C 2 mm flow cell

Detector: DAD, 270 nm

By reducing the flow rate to 2.0 mL/min, the pressure was kept to less than 400 bar improving the separation of a late-eluting peak pair (highlighted) with only a minor increase in analysis time. This separation can be achieved using HPLC or, if a higher flow rate is desired, a UHPLC.



1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol

Poroshell 120 EC-C18 for fast UHPLC separations

Column: **Poroshell 120 EC-C18**
695975-302
3.0 x 100 mm, 2.7 µm

Mobile Phase: 65% A: 0.2% Formic acid
35% B: Methanol
Isocratic

Flow Rate: Varies

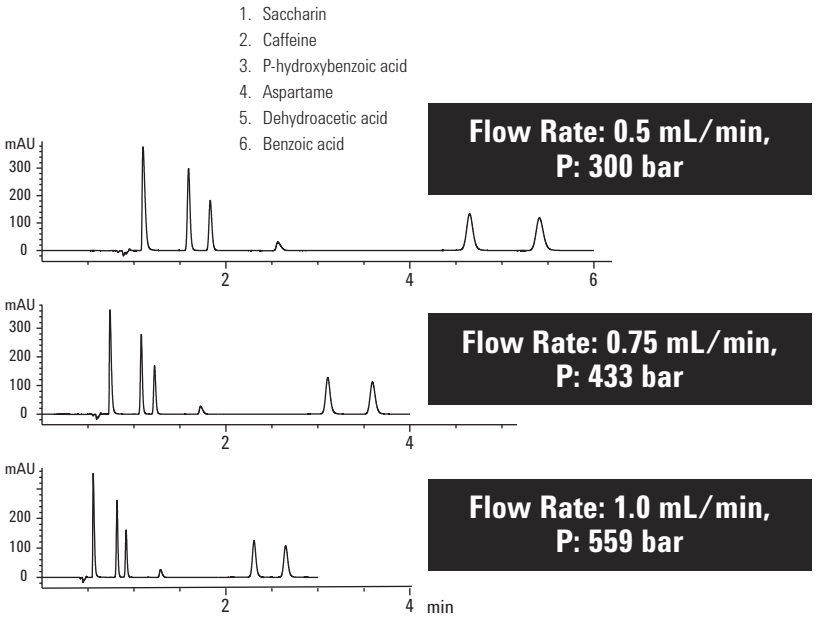
Temperature: 26 °C

Detector: Sig = 220, 4 nm, Ref = Off

This example shows a fast separation using a mobile phase that generates higher pressures. In the top chromatogram, a 3.0 mm id column was used, with a flow rate of 0.5 mL/min and a pressure below 400 bar – making this a typical LC separation.

Although the top separation was fast (just under 6 minutes), the middle and bottom chromatograms show that you can reduce run times to under 3 minutes by increasing the flow rate. These faster analyses will take your pressure to 400-560 bar; look to the Agilent 1200 Infinity Series flexible upgrade options to help you take advantage of UHPLC capabilities.

More viscous solvents like methanol can be used at HPLC or UHPLC pressures.



TIPS & TOOLS

For a full listing of our LC capillary portfolio, turn to pages 16-46.

ZORBAX Rapid Resolution High Definition (RRHD) 1.8 µm

- High pressure (1200 bar) columns for optimum results with the 1290 Infinity LC or other UHPLC instruments
- 1.8 µm particles deliver maximum resolution for the most defined separations
- Available in 12 ZORBAX phases, including Eclipse Plus C18 for superior peak shape, ZORBAX StableBond C18 for low pH stability, Bonus-RP, Eclipse PAH, Eclipse Plus Phenyl-Hexyl and Extend-C18
- Also available in HILIC Plus
- Achieve the same selectivity on 3.5 and 5 µm ZORBAX columns with the same bonded phase for compatibility with any LC

ZORBAX Rapid Resolution High Definition (RRHD) columns are an expansion of the ZORBAX 1.8 µm particle column line. The new RRHD columns use improved packing processes to achieve stability up to 1200 bar for use with the Agilent 1290 Infinity LC or other UHPLC instruments. RRHD 1.8 µm columns are available in 50, 100 and 150 mm lengths for fast or high resolution – truly high definition – separations of your most complex samples.

ZORBAX RRHD Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Endcapped	Temp Limit
ZORBAX Eclipse Plus C18	95Å	160 m^2/g	2.0-9.0	Double	60 °C
ZORBAX Eclipse Plus C8	95Å	160 m^2/g	2.0-9.0	Double	60 °C
ZORBAX Eclipse Plus Phenyl-Hexyl	95Å	160 m^2/g	2.0-9.0	Double	60 °C
ZORBAX Eclipse XDB-C18	80Å	180 m^2/g	2.0-9.0	Double	60 °C
ZORBAX Extend-C18	80Å	180 m^2/g	2.0-11.5**	Double	60 °C
ZORBAX Bonus RP	80Å	180 m^2/g	2.0-9.0	Triple	60 °C
ZORBAX StableBond SB-C18	80Å	180 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond SB-C8	80Å	180 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond SB-Phenyl	80Å	180 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond SB-CN	80Å	180 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond SB-Aq	80Å	180 m^2/g	1.0-8.0*	No	80 °C
ZORBAX Eclipse PAH	95Å	160 m^2/g	2.0-8.0	No	60 °C
ZORBAX HILIC Plus	95Å	160 m^2/g	0.0-8.0	No	60 °C
ZORBAX StableBond 300SB-C8	300Å	45 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond 300SB-C18	300Å	45 m^2/g	1.0-8.0*	No	80 °C
ZORBAX StableBond 300SB-C3	300Å	45 m^2/g	1.0-8.0*	No	80 °C
ZORBAX 300-Diphenyl	300Å	45 m^2/g	1.0-8.0*	No	80 °C

* StableBond columns are designed for optimal use at low pH. At pH >6, highest column stability for all silica based columns is obtained by operating at temperatures <40 °C and using lower buffer concentrations – 10-20 mM or organic buffers. 300SB-C18 may be used up to 90 °C. For pH 6-8, select the Eclipse Plus C18 column.

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

ZORBAX Rapid Resolution High Definition (RRHD) 1.8 µm Columns

Separation of licorice root on RRHD columns

Column A: **ZORBAX RRHD SB-C18**
857700-902
2.1 x 50 mm, 1.8 µm

Column B: **858700-902**
2.1 x 100 mm, 1.8 µm

Column C: **859700-902**
2.1 x 150 mm, 1.8 µm

Mobile Phase: 10-100% B/30 min
A: 0.1% Formic acid (fa)
B: Acetonitrile with 0.1% fa

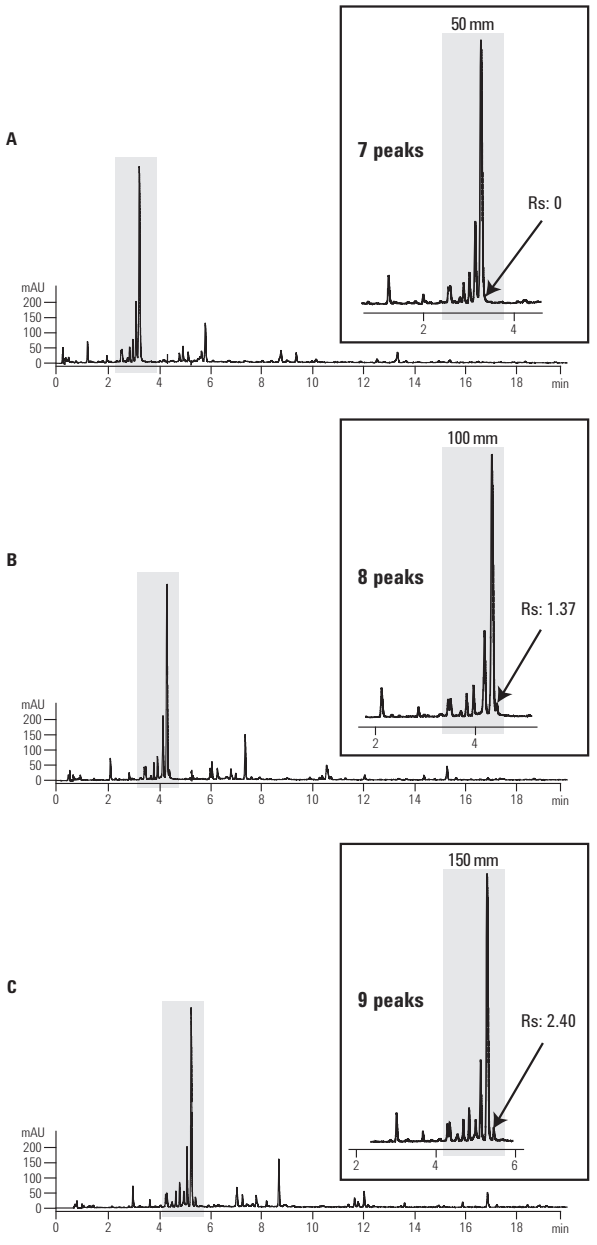
Flow Rate: F = 0.4 mL/min

Gradient: 30 minute gradient on each length

Temperature: Ambient

Detector: 280 nm UV

Instrument: 1290 Infinity LC



Sub 1 minute separations with RRHD columns

Column: **ZORBAX RRHD SB-C18**
857700-902
2.1 x 50 mm, 1.8 µm

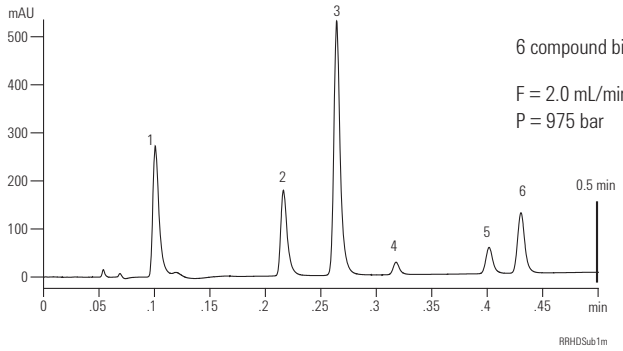
Gradient: H_2O (0.05% trifluoroacetic acid)/10-40% ACN/1 min

Temperature: 60 °C

Injection Volume: 0.5 µL x 100 ppm each

Detector: UV, 275 nm

Data Rate: 160 Hz



6 compound biocide mix in 0.5 min

F = 2.0 mL/min
P = 975 bar

1. 2-methyl-4-isothiazolin-3-one
2. 5-chloro-2-methyl-4-isothiazolin-3-one
3. Carbendazim
4. Benzisothiiazol-3(2H)-one
5. 2-phenoxyethanol
6. Methylparaben

New levels of sensitivity and resolution

Column A: **ZORBAX RRHD Eclipse Plus C18**
959758-302
3.0 x 100 mm, 1.8 µm

Column B: **ZORBAX RRHD Eclipse Plus C18**
959757-302
3.0 x 50 mm, 1.8 µm

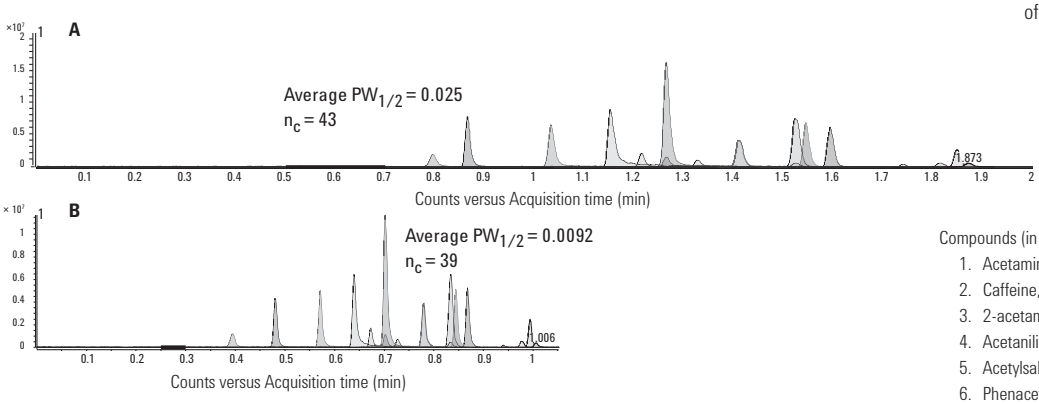
Ion Source: 360 °C, 12 L/min. 50 psi, 3500 V.

Mobile Phase: A: 0.2% Formic acid in water
B: ACN

Temperature: Ambient, no temperature control (approx 24 °C)

Detector: Agilent 1290 Infinity LC with 6410 MS/MS

Sample: 20 µL (10 µL for 50 mm column) of 1 µg/mL standard



By transferring your method to an Agilent RRHD column, you can enhance resolution for difficult analyses – allowing you to save time by using shorter columns without compromising performance.

The RRHD column saves analytical time without sacrificing performance.

Compounds (in elution order) with identifying mass:

1. Acetaminophen, m/z 109
2. Caffeine, m/z 194
3. 2-acetamidophenol, m/z 109
4. Acetanilide, m/z 135
5. Acetylsalicylic acid, m/z 120
6. Phenacetin, m/z 179
7. Salicylic acid, m/z 120
8. Sulindac, m/z 356
9. Piroxicam, m/z 332
10. Tolmetin, m/z 257
11. Ketoprofen, m/z 254
12. Diflunisal, m/z 332
13. Diclofenac, m/z 235
14. Celecoxib, m/z 351
15. Ibuprofen, m/z 160

Selectivity comparison: C18 columns

Column A: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column B: **ZORBAX RRHD Eclipse XDB-C18**
981758-902
2.1 x 100 mm, 1.8 µm

Column C: **ZORBAX RRHD Extend-C18**
758700-902
2.1 x 100 mm, 1.8 µm

Column D: **ZORBAX RRHD SB-C18**
858700-902
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% HCOOH in H_2O (30%)
B: 0.1% HCOOH in CH_3CN (70%)

Flow Rate: 1 mL/min, isocratic

Temperature: 30 °C

Sample: 1 µL

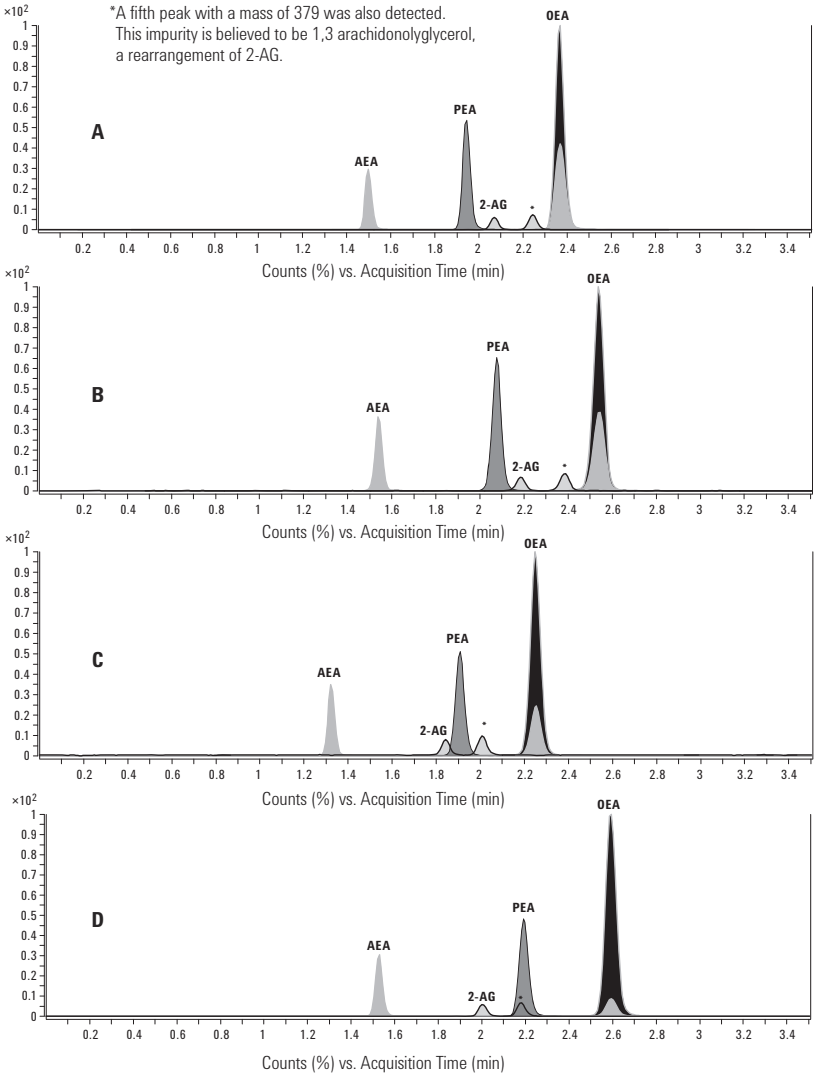
MS2 Scan: 290-390, ESI positive mode, scan time: 500, fragmentor: 135 V; drying gas: 12 L/min, 325 °C; nebulizer pressure: 35 psig; capillary voltage: 3000

Selectivity differences are due to subtle, yet important variations, such as bonding type, endcapping, or the amount and type of silanols on the silica. Other factors that influence selectivity include mobile phase composition, temperature, and pH. (Note that these factors are identical in the following example.)

Here we compared the selectivity of four Agilent ZORBAX RRHD C18 columns using an endocannabinoid analysis method.

1. Anadamine (AEA), m/z 348
2. Palmitoylethanolamide (PEA), m/z PEA
3. 2-arachinoylglycerol (2-AG), m/z 379*
4. Oleoylethanolamide (OEA), m/z 326

*A fifth peak with a mass of 379 was also detected. This impurity is believed to be 1,3 arachidonolyglycerol, a rearrangement of 2-AG.



TIPS & TOOLS

For full details, see Agilent publication 5990-7166EN, **www.agilent.com/chem/library**

Selectivity comparison: Phenyl and other columns

Column A: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column B: **ZORBAX RRHD Eclipse Plus Phenyl-Hexyl**
959758-912
2.1 x 100 mm, 1.8 µm

Column C: **ZORBAX RRHD SB-Aq**
858700-914
2.1 x 100 mm, 1.8 µm

Column D: **ZORBAX RRHD SB-Phenyl**
858700-912
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 5% HCOOH in H_2O
B: CH_3CN

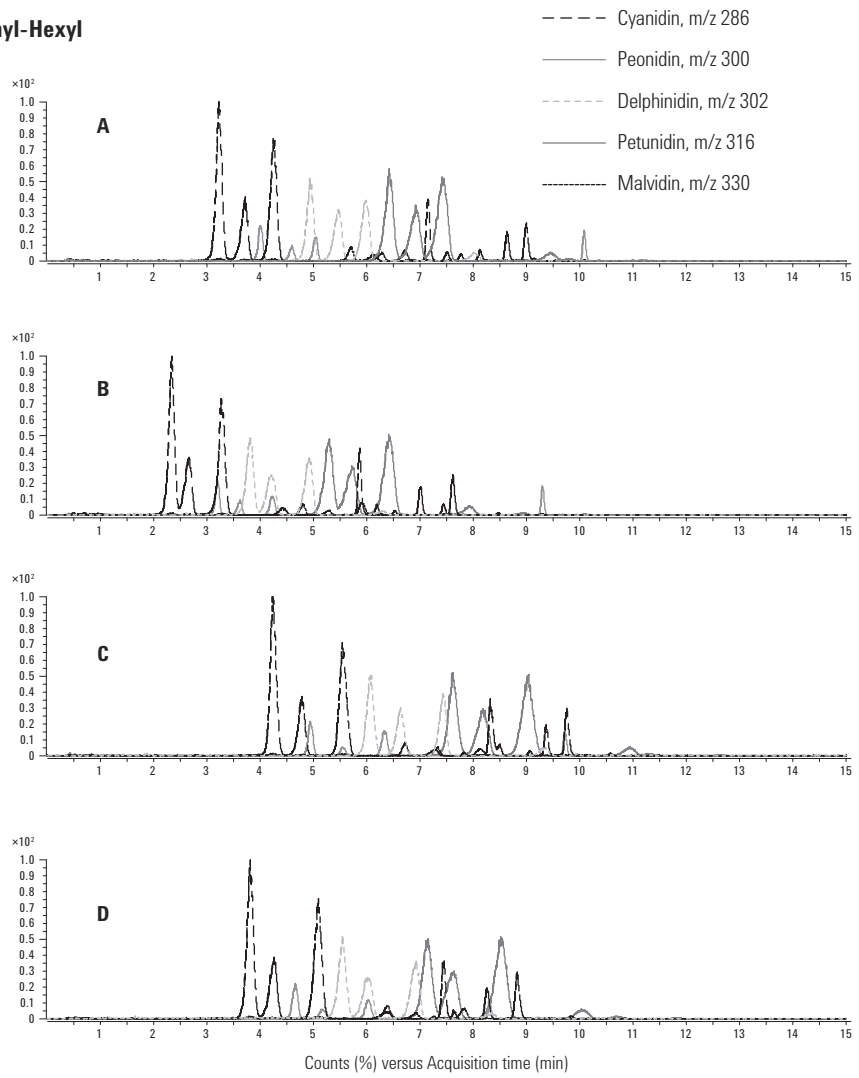
Flow Rate: 0.65 mL/min

Gradient: 10-50% B in 15 min

Temperature: 30 °C

MS2 Scan: ESI +, 200-1000

Extracted ion chromatograms from LC/MS scan data of blueberry anthocyanins.



TIPS & TOOLS

For full details, see Agilent publication 5990-8470EN, **www.agilent.com/chem/library**

Rapid Resolution High Definition (RRHD) Columns for High Pressure Use (Maximum Pressure: 1200 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse Plus Phenyl-Hexyl USP L11	Eclipse PAH USP L1
	Solvent Saver RRHD, 1200 bar	3.0 x 150	1.8	959759-302	959759-306		
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	959758-302	959758-306	959758-312	959758-318
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	959757-302	959757-306	959757-312	959757-318
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-901			
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	959759-902	959759-906	959759-912	959763-918
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	959758-902	959758-906	959758-912	959764-918
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	959757-902	959757-906	959757-912	959741-918
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-901			

Rapid Resolution High Definition (RRHD) Columns for High Pressure Use (Maximum Pressure: 1200 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-CN USP L10	SB-Phenyl USP L11	SB-Aq
	Solvent Saver RRHD, 1200 bar	3.0 x 150	1.8	859700-302	859700-306			
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	858700-302	858700-306	858700-305	858700-905	858700-314
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	857700-302	857700-306	857700-305	857700-312	857700-314
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-902	823750-904			
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	859700-902	859700-906	859700-905	859700-912	859700-914
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	858700-902	858700-906	858700-905	858700-912	858700-914
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	857700-902	857700-906	857700-905	857700-912	857700-914
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-902	821725-904			

Rapid Resolution High Definition (RRHD) Columns for High Pressure Use (Maximum Pressure: 1200 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	Extend-C18 USP L1	Eclipse XDB-C18 USP L1	Bonus-RP USP L60	HILIC Plus
	Solvent Saver RRHD, 1200 bar	3.0 x 150	1.8	759700-302	981759-302		
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	758700-302	981758-302		959758-301
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	757700-302	981757-302		959757-301
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8		823750-903		
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	759700-902	981759-902	859768-901	959759-901
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	758700-902	981758-902	858768-901	959758-901
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	757700-902	981757-902	857768-901	959757-901
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8		821725-903		

ZORBAX RRHD columns are also available in 300Å configurations for biomolecules. Turn to page 364.

ZORBAX RRHD 300-HILIC will be available in 2013.

ZORBAX Rapid Resolution High Throughput (RRHT) 1.8 µm

- 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 µm materials
- Reduce analysis time by up to 95%
- Develop HPLC methods more quickly
- Securely transfer conventional methods with over 140 RRHT column choices
- Analyze complex samples on shorter columns faster and maximize peak capacity
- Matching selectivity in 3.5, 5 and 7 µm particle sizes for complete method scalability
- Short (50 mm long and less) column can be used on some conventional LCs

ZORBAX Rapid Resolution High Throughput (RRHT) 1.8 µm Columns

Agilent 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 140 RRHT column choices, including the high performance ZORBAX Eclipse Plus and many other ZORBAX column choices (Eclipse XDB, StableBond, Extend, Bonus-RP), 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 columns 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 Agilent1260 Infinity LC System up to this high pressure limit. In addition, the shorter columns can be used on many other LC's, including the Agilent 1200 Rapid Resolution LC System.

Rapid Resolution HT (RRHT) provides double the efficiency of Rapid Resolution columns

Column A: **ZORBAX Rapid Resolution SB-C18**
835975-902
4.6 x 50 mm, 3.5 µm

Column B: **ZORBAX RRHT SB-C18**
827975-902
4.6 x 50 mm, 1.8 µm

Mobile Phase: 25% Water, 75% MeOH

Flow Rate: 1.5 mL/min

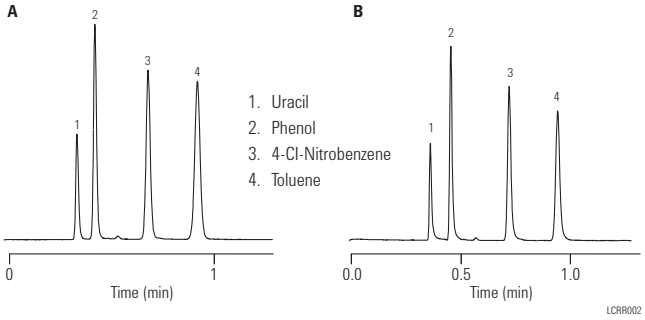
Temperature: Ambient

Detector: UV, 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.

Plates (N)	
1.	3476
2.	4585
3.	5673
4.	6180

Plates (N)	
1.	6560
2.	8958
3.	11508
4.	12266



Increase peak capacity with RRHT columns

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

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

Mobile Phase: A: H_2O
B: ACN

Peak capacity: A: 461
B: 343

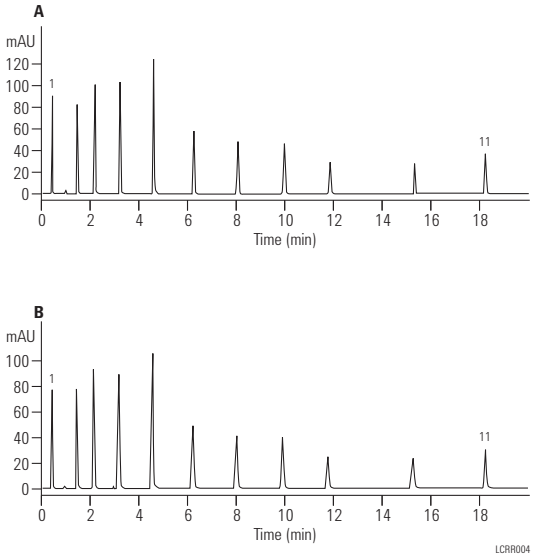
Flow Rate: 0.5 mL/min

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

Temperature: 40 °C

Detector: UV, 254 nm

Sample: Alkylphenones



Reduce analysis time dramatically with Rapid Resolution HT columns

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

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

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

Column D: **ZORBAX Eclipse XDB-C18**
935967-902
4.6 x 50 mm, 3.5 µm

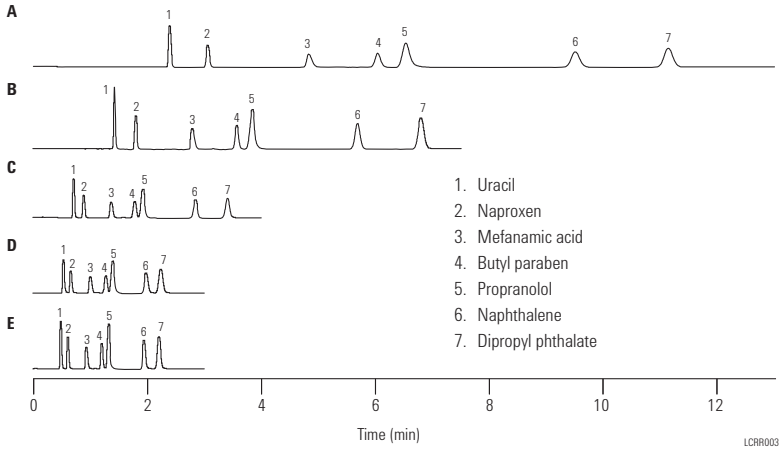
Column E: **Eclipse RRHT XDB-C18**
925975-902
4.6 x 50 mm, 1.8 µm

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

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 254 nm



This figure shows the dramatic reduction in analysis time made 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.

Long lifetime of RRHT columns at elevated temperatures

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

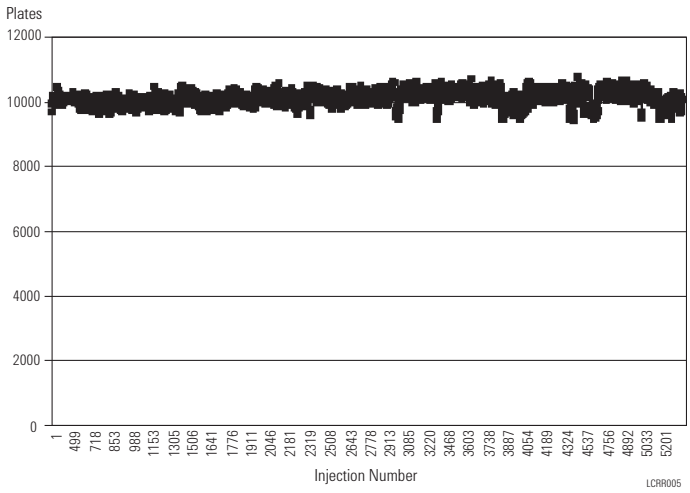
Mobile Phase: A: 60% H_2O
B: 40% ACN

Flow Rate: 1 mL/min

Temperature: 80 °C

Detector: UV, 254 nm

Sample: QC test mix



Comparison of efficiencies – Rapid Resolution High Definition (RRHD)/RRHT (1.8 µm) and Rapid Resolution (3.5 µm) columns

Column Length (mm)	Poroshell 120	Resolving Power N (3.5 µm)*	Resolving Power N (1.8 µm)
High Resolution			
150	32,000	21,000	32,500
100	21,000	14,000	24,000
75	16,000	10,500	17,000**
Ultra Fast			
50	11,000	7,000	12,000
30	5,500	4,200	6,000
20	—	—	3,500
15	—	2,100	2,500
Resolution α $N^{1/2}$			

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

**Available as a custom column

Data is based on 4.6 mm id columns

TIPS & TOOLS

The LC Rack from Agilent can help you reduce capillary lengths and minimize extra-column volume. It also protects your instrument and enables you to switch out modules as needed.

Agilent rack for LC systems, 5001-3726

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

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse Plus Phenyl-Hexyl USP L11	Eclipse PAH USP L1	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	959964-912	959964-918	928975-902		728975-902
	Rapid Resolution HT, 600 bar	4.6 x 75	1.8	959951-902						
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	959941-902	959941-906	959941-912	959941-918	927975-902	927975-906	727975-902
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	959931-902	959931-906	959931-912	959931-918	924975-902	924975-906	724975-902
	Rapid Resolution HT, 600 bar	4.6 x 20	1.8					926975-902	926975-906	726975-902
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	1.8	820750-901				820750-903		
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	959964-302	959964-306	959964-312		928975-302		728975-302
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	959941-302	959941-306	959941-312		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
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-901				823750-903		
	Narrow Bore RRHT, 600 bar	2.1 x 150	1.8	959794-902						
	Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	959764-902	959764-906	959764-912	959764-918	928700-902	928700-906	728700-902
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	959741-902	959741-906	959741-912	959741-918	927700-902	927700-906	727700-902
	Narrow Bore RRHT, 600 bar	2.1 x 30	1.8	959731-902	959731-906	959731-912		924700-902	924700-906	724700-902
	Narrow Bore RRHT, 600 bar	2.1 x 20	1.8					926700-902	926700-906	726700-902
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-901				821725-903		

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

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-Phenyl USP L11	SB-CN USP L10	SB-Aq	Rx-SIL USP L3	Bonus-RP USP L60
	Rapid Resolution HT, 600 bar	4.6 x 150	1.8	829975-902	829975-906	829975-912	829975-905	829975-914		
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	828975-902	828975-906	828975-912	828975-905	828975-914	828975-901	828668-901
	Rapid Resolution HT, 600 bar	4.6 x 75	1.8		830975-906					830668-901
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	827975-902	827975-906	827975-912	827975-905	827975-914	827975-901	827668-901
	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					
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	1.8	820750-902	820750-904					
	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	828975-301	828668-301
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	827975-302	827975-306	827975-312	827975-305	827975-314	827975-301	827668-301
	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					
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-902	823750-904					
	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	828700-901	828768-901
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	827700-902	827700-906	827700-912	827700-905	827700-914	827700-901	827768-901
	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					
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-902	821725-904					

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

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	SB-C18 USP L1	SB-C8 USP L7	Extend-C18 USP L1
	Rapid Resolution HT, 400 bar	4.6 x 50	1.8	922975-902	922975-906	922975-902	822975-906	722975-902
	Rapid Resolution HT, 3/pk, 400 bar	4.6 x 50	1.8	922975-932		922975-932		
	Narrow Bore RRHT, 400 bar	2.1 x 50	1.8	922700-902		922700-902		
	Narrow Bore RRHT, 3/pk, 400 bar	2.1 x 50	1.8	922700-932		922700-932		
Rapid Resolution HT Cartridges (require hardware kit 820555-901)								
RR	Rapid Resolution HT Cartridge	4.6 x 50	1.8	925975-902		825975-902		
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 50	1.8	925975-932		825975-932		
RR	Rapid Resolution HT Cartridge	2.1 x 50	1.8	925700-902		825700-902		
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 50	1.8	925700-932		825700-932		
RR	Rapid Resolution HT Cartridge	4.6 x 30	1.8	923975-902		823975-902		
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 30	1.8	923975-932		823975-932		
RR	Rapid Resolution HT Cartridge	2.1 x 30	1.8	923700-902		823700-902		
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 30	1.8	923700-932		823700-932		
RR	Rapid Resolution HT Cartridge	4.6 x 15	1.8	921975-902		821975-902		
RR	Rapid Resolution HT Cartridge, 3/pk	4.6 x 15	1.8	921975-932		821975-932		
RR	Rapid Resolution HT Cartridge	2.1 x 15	1.8	921700-902		821700-902		
RR	Rapid Resolution HT Cartridge, 3/pk	2.1 x 15	1.8	921700-932		821700-932		
RR	Hardware Kit for RR and RRHT Cartridges			820555-901		820555-901		

UHPLC Guard, 1200 bar, 821725-903

Agilent Fast Guards for UHPLC

- High performance guard columns for Fast LC columns
- Two formats – one for Poroshell 120 columns, stable to 600 bar, RRHD columns, 1.8 µm (stable to 1200 bar), and RRHT columns, 1.8 µm (stable to 600 bar)

Agilent UHPLC Guards are high performance guards designed by Agilent for its Fast LC columns families. Agilent UHPLC Guards use easy-to-install hardware that fits directly on the end of the column; no extra hardware is needed. They are sold in packages of three.

Agilent UHPLC Guards extend the lifetime of analytical columns without diminishing performance.

Fast Guards for UHPLC

ZORBAX RRHD columns, 1.8 µm (1200 bar), and ZORBAX RRHT columns, 1.8 µm (600 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse XDB-C18 USP L1	SB-C18 USP L1	SB-C8 USP L7
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-901	821725-903	821725-902	821725-904
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-901	823750-903	823750-902	823750-904
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	1.8	820750-901	820750-903	820750-902	820750-904

Poroshell 120 columns, 2.7 µm (600 bar)

Hardware	Description	Size (mm)	Particle Size (µm)	EC-C18 USP L1	EC-C8 USP L7	SB-C18 USP L1	Phenyl-Hexyl USP L11
UG	UHPLC Guard, 600 bar, 3/pk	2.1 x 5	2.7	821725-911	821725-913	821725-912	821725-914
UG	UHPLC Guard, 600 bar, 3/pk	3.0 x 5	2.7	823750-911	823750-913	823750-912	823750-914
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	2.7	820750-911	820750-913	820750-912	820750-914

TIPS & TOOLS

Learn about Fast Guards for UHPLC – an easy way to extend the life of your analytical Fast LC column without losing performance. **www.agilent.com/chem/fastguardsvideo**

Other Columns for Reversed-Phase Analytical HPLC

Achieve excellent peak shape and resolution every time – leveraging the industry's broadest selection of reversed-phase columns

Whether you are using Fast LC or working with more conventional HPLC applications, Agilent's LC family offers you a range of phases and selectivities to help you perfect your separation.

The ZORBAX Family of phases scales readily to Fast LC columns in the Rapid Resolution High Throughput (RRHT) and Rapid Resolution High Definition (RRHD) families and Poroshell 120 columns, see previous section, page 227.

In this section, we'll provide overviews of other key analytical columns from Agilent:

ZORBAX Rapid Resolution, 3.5 µm, configurations are an ideal choice for initial method development, providing increased sample throughput for any application when compared to 5 µm columns.

ZORBAX Solvent Saver 3.0 mm id column configurations provide 60% mobile phase reduction over 4.6 mm id columns.

ZORBAX Eclipse Plus HPLC columns are designed to reliably produce superior peak shapes for basic compounds, and are available across all ZORBAX column configurations.

More than 13 additional ZORBAX phases including StableBond, Eclipse PAH, Eclipse XDB, ZORBAX Rx, Extend-C18, Bonus-Rx and Original ZORBAX columns – in total, more than 1400 configurations for reliable scalability and method transfer.

ZORBAX Method Development kits contain three columns for the price of two! Each as a different bonded phase for optimizing selectivity.

ZORBAX Method Validation kits – choose as many columns as you need (or as few) to make method validation easier and less expensive.

Pursuit, Pursuit XRs and Pursuit XRs Ultra columns provide alternate selectivities to the ZORBAX family.

Polaris Columns provide polar-modified phases for routine polar applications.

Other Columns for Reversed-Phase Analytical HPLC.

ZORBAX Eclipse Plus Columns

ZORBAX Eclipse Plus

- The ideal column for method development – excellent results for a wide range of compounds
- High level of performance – peak shape, efficiency, resolution, and lifetime – with all sample types: acids, bases and neutrals
- Superior reproducibility with more rigorous QA/QC testing
- Improved, patented silica manufacturing with start-to-finish product control
- Available in 1.8, 3.5, and 5 µm particle sizes for all analytical, high resolution, and fast LC analyses

Agilent ZORBAX Eclipse Plus columns provide the ultimate in performance for silica-based columns. Peak shape is excellent for the most challenging basic compounds, improving efficiency and resolution with these sample types. These results are achieved by improvements in the silica manufacturing and bonding technology, which is completely controlled by Agilent.

Because of their high level of performance, Eclipse Plus columns are the ideal first choice for method development of all samples. If you need to achieve fast method development and superior productivity, then choose a column with high-resolution 1.8 µm particles. For standard methods, conventional 5 µm and Rapid Resolution 3.5 µm columns are your best choice. With all particle sizes, easy method transfer is possible.

With more rigorous QA and QC testing, column lot-to-lot reproducibility is also improved, resulting in long-term reliable results for all analyses.

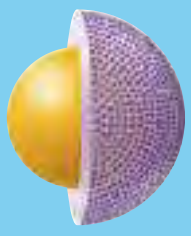
Column Specifications

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	9%
ZORBAX Eclipse Plus C8	95Å	160 m^2/g	60 °C	2.0-9.0	Double	7%
ZORBAX Eclipse PAH	95Å	160 m^2/g	60 °C	2.0-8.0	No	14%
ZORBAX Eclipse Plus Phenyl-Hexyl	95Å	160 m^2/g	60 °C	2.0-8.0	Double	9%

Specifications represent typical values only.

*Column lifetime will be reduced significantly at pH >7 and temperature >40 °C. At pH 6-9, highest column stability for all silica based columns is obtained by operating at temperatures <40 °C and using lower buffer concentrations in range of 0.01-0.02 M, especially with phosphate and carbonate buffers.

TIPS & TOOLS



The EC-C18, EC-C8 and Phenyl-Hexyl phases on Poroshell 120 are very similar to Eclipse Plus C18, Eclipse Plus C8 and Eclipse Plus Phenyl-Hexyl phases.

Turn to page 228.

ZORBAX Eclipse Plus: Best peak shape in the industry without tailing

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

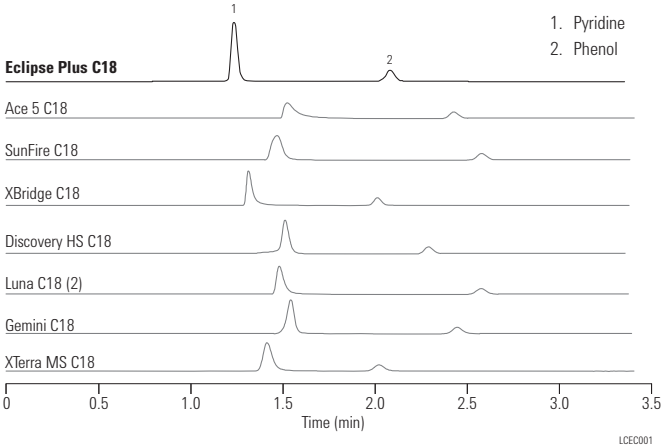
Mobile Phase: A: 60% Water
B: 40% Acetonitrile

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Pyridine, Phenol



Peak shape and efficiency are better with ZORBAX Eclipse Plus

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

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

Mobile Phase: A: 0.1% Formic acid
B: 0.1% Formic acid in ACN

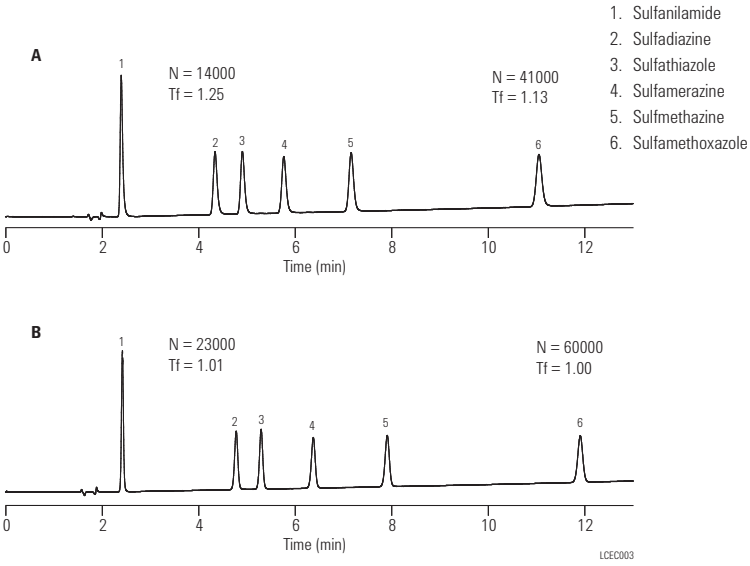
Flow Rate: 1.0 mL/min

Gradient: 0.0 min 10% B
15 min 30% B

Temperature: 40 °C

Detector: UV, 254 nm

Sample: Sulfonamides



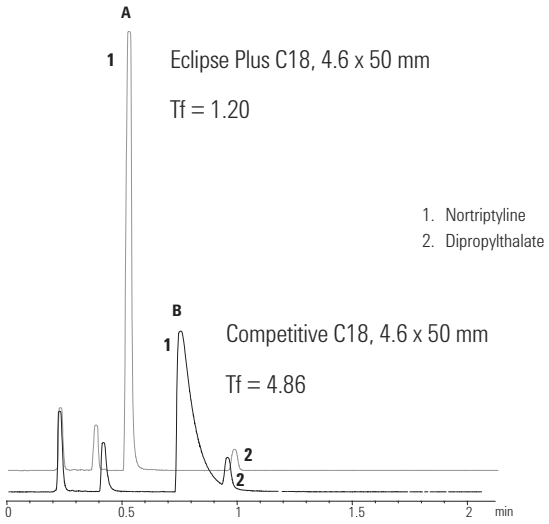
Eliminate tailing and maximize resolution with Eclipse Plus Columns

Column A: **Eclipse Plus C18, 4.6 x 50 mm**

Column B: **Competitive C18, 4.6 x 50 mm**

Mobile Phase: 65% ACN:35% 25 mM phosphate buffer (pH 7.4)

Superior peak shape and better selectivity with Eclipse Plus means more resolution, easier quantitation and better results in your separations.



Eclipse Plus C18 vs. C8

Column A: **Eclipse Plus C18 4.6 x 50 mm, 5 µm**

Column B: **Eclipse Plus C8 4.6 x 50 mm, 5 µm**

Mobile Phase: Water: acetonitrile (30:70)

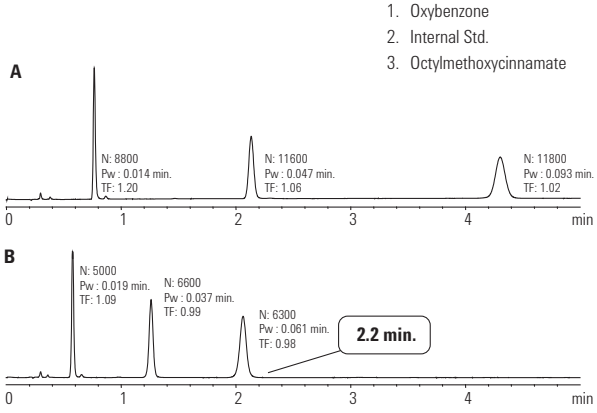
Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: UV, 230 nm

Sample: Lip balm extract in ACN (melted at 100 °C, cooled and 0.45 µm filtered)

Less retention can save significant time – the C8 is a good choice here.



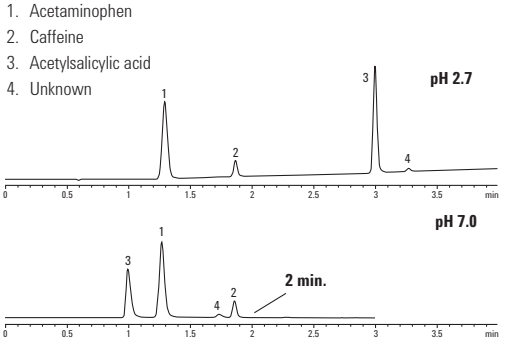
Rapid analysis of an analgesic tablet, selectivity differences at pH 2 and pH 7

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

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

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 100 mm, 5 µm

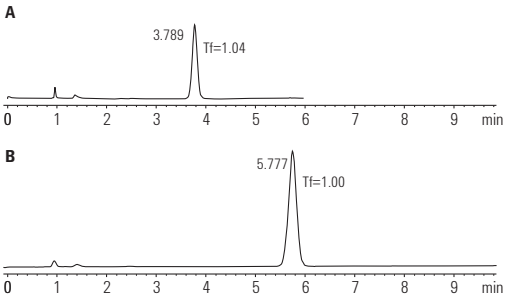
Column B: **Eclipse Plus C18**
959996-902
4.6 x 100 mm, 5 µm

Mobile Phase: 80% Methanol 8 mM (total) K_2HPO_4 pH 7

Flow Rate: 1.0 mL/min

Detector: UV, 215 nm

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.

Fast and ultra-fast analysis of basic compounds on Eclipse Plus

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

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

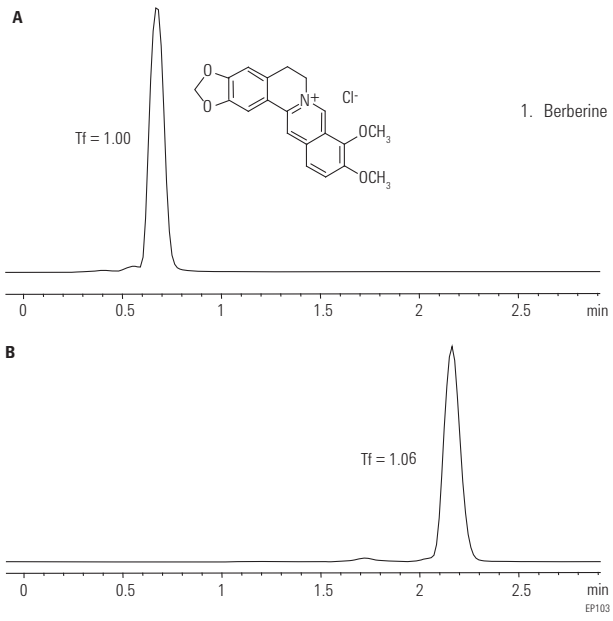
Mobile Phase: A: 50% 8 mM K_2HPO_4, pH 7
B: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Berberine, 0.4 mg/mL, 2 µL



ZORBAX Eclipse Plus Columns

ZORBAX Eclipse Plus

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse Plus Phenyl-Hexyl USP L11	Eclipse PAH USP L1
	Analytical	4.6 x 250	5	959990-902	959990-906	959990-912	959990-918
	Analytical	4.6 x 150	5	959993-902	959993-906	959993-912	959993-918
	Analytical	4.6 x 100	5	959996-902	959996-906	959996-912	959996-918
	Analytical	4.6 x 50	5	959946-902	959946-906		
	Rapid Resolution	4.6 x 150	3.5	959963-902	959963-906	959963-912	959963-918
	Rapid Resolution	4.6 x 100	3.5	959961-902	959961-906	959961-912	959961-918
	Rapid Resolution	4.6 x 75	3.5	959933-902	959933-906	959933-912	
	Rapid Resolution	4.6 x 50	3.5	959943-902	959943-906	959943-912	959943-918
	Rapid Resolution	4.6 x 30	3.5	959936-902	959936-906	959936-912	
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	959964-902	959964-906	959964-912	959964-918
	Rapid Resolution HT, 600 bar	4.6 x 75	1.8	959951-902			
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	959941-902	959941-906	959941-912	959941-918
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	959931-902	959931-906	959931-912	959931-918
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	1.8	820750-901			
	Solvent Saver	3.0 x 250	5				959990-318
	Solvent Saver	3.0 x 150	5	959993-302	959993-306		
	Solvent Saver Plus	3.0 x 150	3.5	959963-302	959963-306	959963-312	
	Solvent Saver Plus	3.0 x 100	3.5	959961-302	959961-306	959961-312	
	Solvent Saver RRHD, 1200 bar	3.0 x 150	1.8	959759-302	959759-306		
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	959758-302	959758-306		
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	959757-302	959757-306		
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	959964-302	959964-306	959964-312	
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	959941-302	959941-306	959941-312	

(Continued)

Agilent HILIC Plus uses the same manufacturing processes as the Eclipse Plus family.
See information about ZORBAX HILIC Plus on page 324.

ZORBAX Eclipse Plus

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse Plus C18 USP L1	Eclipse Plus C8 USP L7	Eclipse Plus Phenyl-Hexyl USP L11	Eclipse PAH USP L1
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-901			
	Narrow Bore	2.1 x 250	5				959790-918
	Narrow Bore	2.1 x 150	5	959701-902	959701-906	959701-912	959701-918
	Narrow Bore	2.1 x 50	5	959746-902	959746-906		
	Narrow Bore RR	2.1 x 150	3.5	959763-902	959763-906	959763-912	
	Narrow Bore RR	2.1 x 100	3.5	959793-902	959793-906	959793-912	959793-918
	Narrow Bore RR	2.1 x 50	3.5	959743-902	959743-906	959743-912	
	Narrow Bore RR	2.1 x 30	3.5	959733-902	959733-906	959733-912	
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	959759-902	959759-906		
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	959758-902	959758-906		
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	959757-902	959757-906		
	Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	959764-902	959764-906	959764-912	959764-918
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	959741-902	959741-906	959741-912	959741-918
	Narrow Bore RRHT, 600 bar	2.1 x 30	1.8	959731-902	959731-906	959731-912	
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-901			
ZGC	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-936	820950-937	820950-938	820950-939
ZGC	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-936	821125-937	821125-938	821125-939
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901

ZORBAX Eclipse PAH Columns

ZORBAX Eclipse PAH

- High resolution separation of 16 PAHs in EPA Method 610
- Extensive range of particle sizes (1.8, 3.5 and 5 µm) and sizes for fast and high resolution separations
- Each batch of material is specifically tested with PAHs for maximum reproducibility under expected operating conditions
- Excellent performance using the high quality, improved silica of Eclipse Plus columns
- Good for applications requiring "shape selectivity" or the separation of geometric isomers

Agilent ZORBAX Eclipse PAH columns are recommended for the separation of polycyclic aromatic hydrocarbons. PAHs are considered priority pollutants and the analysis of these potentially carcinogenic compounds in water, soil and food is of major importance. Eclipse PAH columns separate all 16 PAHS in EPA method 610 quickly and with high resolution.

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range	Endcapped	Carbon Load
ZORBAX Eclipse PAH	95Å	160 m^2/g	60 °C	2.0-8.0	No	14%

Specifications represent typical values only.

High resolution and fast analysis on RRHT Eclipse PAH column

Column: **Eclipse PAH 959941-918 4.6 x 50 mm, 1.8 µm**

Mobile Phase: A: Water; B: Acetonitrile

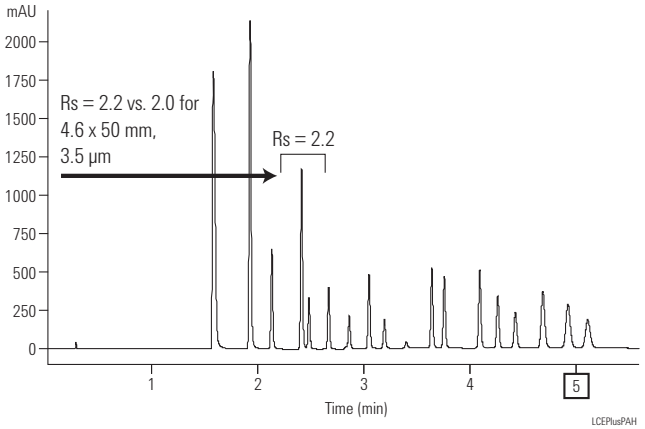
Gradient:

Time (Min)	% B
0.00	40
3.5	100
5.2	100
5.5	40
6.5	40

Flow Rate: 2.0 mL/min

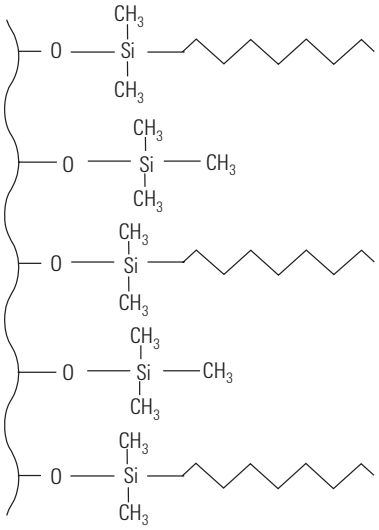
Temperature: 25 °C

Detector: DAD 220, 4 nm No Ref. DAD Stop Time = 6.0 min Stop Time = 7.0



ZORBAX Eclipse PAH

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse PAH USP L1
	Analytical	4.6 x 250	5	959990-918
	Analytical	4.6 x 150	5	959993-918
	Analytical	4.6 x 100	5	959996-918
	Rapid Resolution	4.6 x 150	3.5	959963-918
	Rapid Resolution	4.6 x 100	3.5	959961-918
	Rapid Resolution	4.6 x 50	3.5	959943-918
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	959964-918
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	959941-918
	Rapid Resolution HT, 600 bar	4.6 x 30	1.8	959931-918
	Solvent Saver	3.0 x 250	5	959990-318
	Narrow Bore	2.1 x 250	5	959790-918
	Narrow Bore	2.1 x 150	5	959701-918
	Narrow Bore RR	2.1 x 100	3.5	959793-918
	Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	959764-918
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	959741-918
ZGC	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-939
ZGC	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-939
ZGC	Guard Hardware Kit			820999-901



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 to 7 µm
- Long lifetime with eXtra Dense Bonding and double endcapping

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.

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%

Specifications represent typical values only

*Eclipse XDB columns are designed for operation over a wide pH range.At pH 6-9, highest columns stability for all silica based columns is achieved by operating at temperatures <40 °C and using low buffer concentrations in the range of 0.01-0.02 M.

TIPS & TOOLS

Poroshell 120 EC-CN is very similar to ZORBAX XDB-CN. Page 228

Good peak shape over a wide pH range with ZORBAX Eclipse XDB

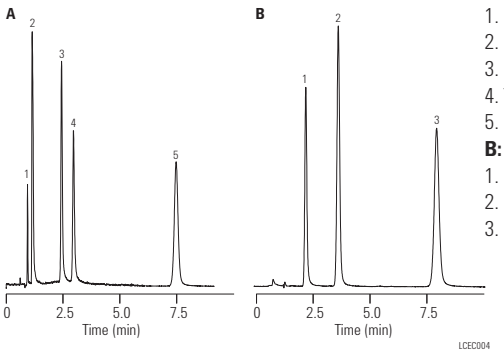
Column: **Eclipse XDB-C8**
993967-906
4.6 x 150 mm, 5 µm

Mobile Phase: A: pH 3.0 75% 25 mM phosphate buffer 25% ACN
B: pH 7.0 90% 20 mM phosphate 10% ACN

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.



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

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

Column stability testing at pH 3 and 60 °C

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

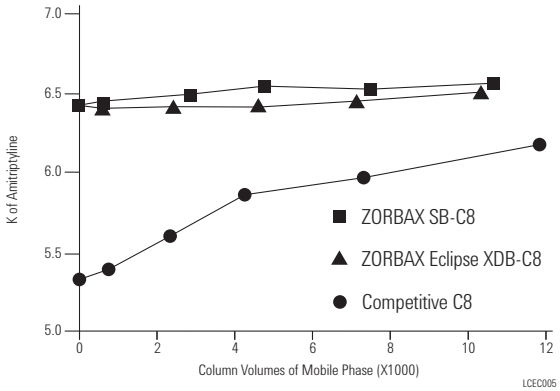
Column: **Eclipse XDB-C8**
993967-906
4.6 x 150 mm, 5 µm

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 an Eclipse endcapped 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 A: **Competitive C8**
SIL-type
After 1826 column volumes

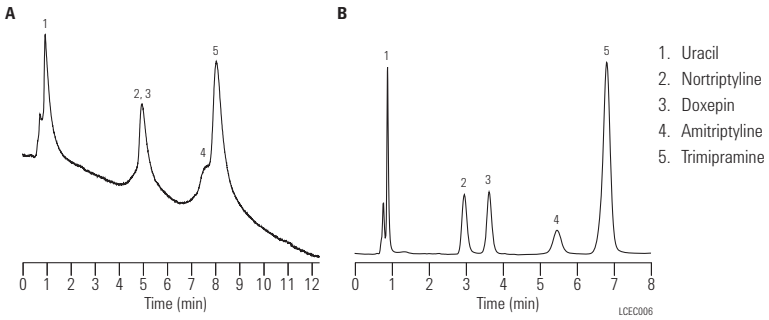
Column B: **Eclipse XDB-C8**
993967-906
4.6 x 150 mm, 5 µm
Sol-type
After 1843 column volumes

Mobile Phase: 60% ACN
40% 250 mM Phosphate Buffer, pH 7.0

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Sample: Tricyclic antidepressants



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 StableBond

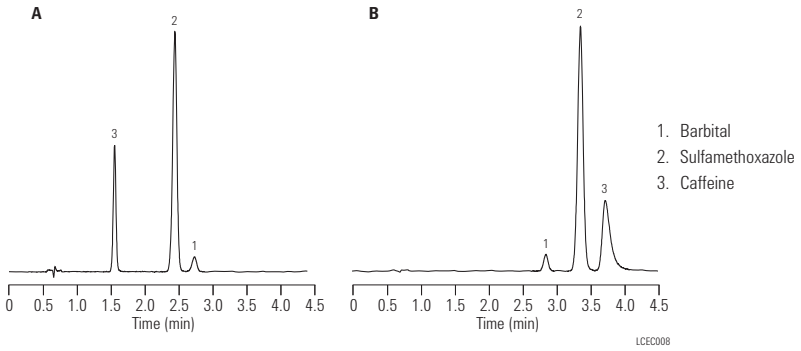
Column A: **Eclipse XDB-C8**
966967-906
4.6 x 75 mm, 3.5 µm

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

Mobile Phase: 70% 25 mM NaH_2PO_4, pH 3.0
30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35 °C



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

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

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

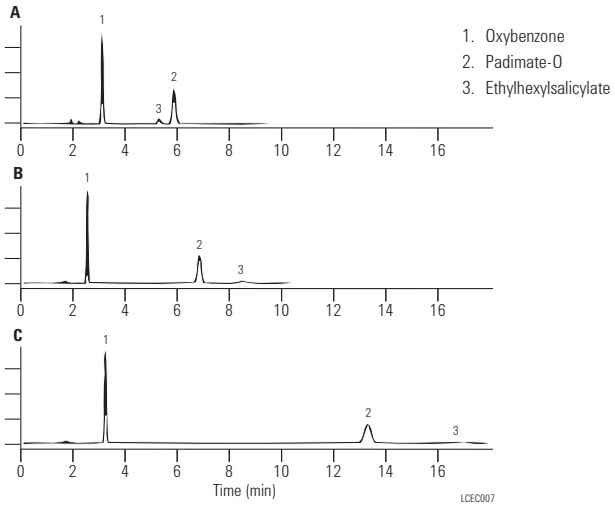
Column C: **Eclipse XDB-C18 963967-902 4.6 x 150 mm, 3.5 µm**

Mobile Phase: 15% H_2O:85% MeOH

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Sample: Sunscreens



1. Oxybenzone
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.

Selectivity for urea pesticides

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

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

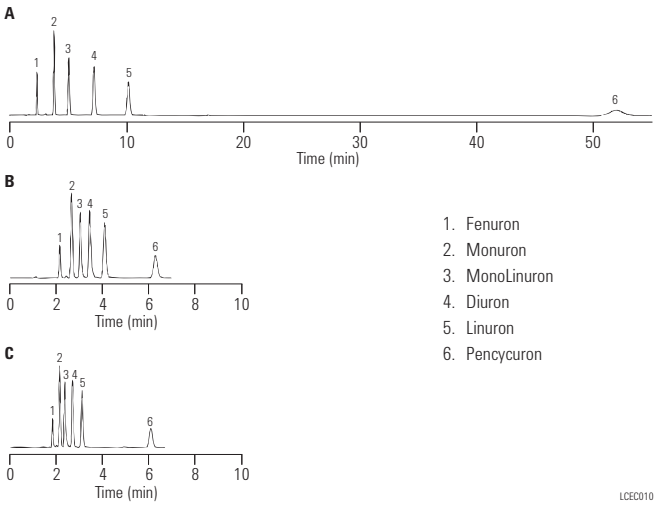
Column C: **Eclipse XDB-C18 993967-902 4.6 x 150 mm, 5 µm**

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



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

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)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Eclipse XDB-Phenyl USP L11	Eclipse XDB-CN USP L10
Standard Columns (no special hardware required)							
	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	4.6 x 30	3.5	934967-902	934967-906		
	Rapid Resolution	4.6 x 20	3.5	932967-902	932967-906		
UG	UHPLC Guard, 1200 bar, 3/pk	4.6 x 5	1.8	820750-903			
	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 RRHD, 1200 bar	3.0 x 150	1.8	981759-302			
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	981758-302			
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	981757-302			
	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		
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-903			
	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		

Unless indicated, column pressure limit is 400 bar.

*These columns are packed with Eclipse XDB-C18, 5 µm.

(Continued)

ZORBAX Eclipse XDB

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7	Eclipse XDB-Phenyl USP L11	Eclipse XDB-CN USP L10
Standard Columns (no special hardware required)							
	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 RR	2.1 x 30	3.5	974700-902	974700-906		
	Narrow Bore RR	2.1 x 20	3.5	972700-902	972700-906		
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	981759-902			
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	981758-902			
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	981757-902			
	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		
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-903			
	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, 3/pk	1.0 x 17	5	5185-5921	5185-5921		
P	Guard Cartridge	9.4 x 15	5	820675-112*	820675-112*	820675-112*	820675-112*
ZGC	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-925	820950-926	820950-927	820950-935
ZGC	Guard Cartridges, 4/pk	2.1 x 12.5	5	821125-926	821125-926	821125-926	821125-935
P	Guard Hardware Kit			840140-901	840140-901	840140-901	840140-901
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)							
PH	PrepHT Cartridge	21.2 x 250	7	977250-102	977250-106		
PH	PrepHT Cartridge	21.2 x 150	7	977150-102	977150-106		
PH	PrepHT Cartridge	21.2 x 150	5	970150-902	970150-906		
PH	PrepHT Cartridge	21.2 x 100	5	970100-902	970100-906		
PH	PrepHT Cartridge	21.2 x 50	5	970050-902	970050-906		
PH	PrepHT Guard Cartridge	17.0 x 7.5	5	820212-925	820212-926		
PH	Guard Cartridge Hardware			820444-901	820444-901		
PH	PrepHT Endfittings, 2/pk			820400-901	820400-901		

Unless indicated, column pressure limit is 400 bar.

*These columns are packed with Eclipse XDB-C18, 5 µm.

ZORBAX Eclipse XDB

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7
Agilent Cartridge Columns (require hardware kit 5021-1845)					
AC	Analytical	4.6 x 250	5	7995118-585	7995108-585
AC	Analytical	4.6 x 150	5	7995118-595	7995108-595
AC	Rapid Resolution	4.6 x 75	3.5	7995118-344	7995108-344
AC	Solvent Saver Plus	3.0 x 75	3.5	7995230-344	
AC	Guard Cartridges, 10/pk	4.0 x 4	5	7995118-504	7995118-504
AC	Cartridge Holder			5021-1845	5021-1845
Standard Columns (no special hardware required)					
	Rapid Resolution HT, 400 bar	4.6 x 50	1.8	922975-902	922975-906
	Rapid Resolution HT, 3/pk, 400 bar	4.6 x 50	1.8	922975-932	
	Narrow Bore RRHT, 400 bar	2.1 x 50	1.8	922700-902	
	Narrow Bore RRHT, 3/pk, 400 bar	2.1 x 50	1.8	922700-932	
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, 400 bar	4.6 x 50	1.8	925975-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	4.6 x 50	1.8	925975-932	
RR	Rapid Resolution HT Cartridge, 400 bar	4.6 x 30	1.8	923975-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	4.6 x 30	1.8	923975-932	

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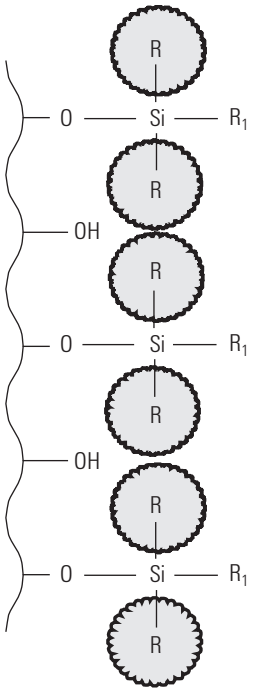
ZORBAX Eclipse XDB

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7
Rapid Resolution HT Cartridges (require hardware kit 820555-901)					
RR	Rapid Resolution HT Cartridge, 400 bar	4.6 x 15	1.8	921975-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	4.6 x 15	1.8	921975-932	
RR	Rapid Resolution HT Cartridge, 400 bar	2.1 x 50	1.8	925700-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	2.1 x 50	1.8	925700-932	
RR	Rapid Resolution HT Cartridge, 400 bar	2.1 x 30	1.8	923700-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	2.1 x 30	1.8	923700-932	
RR	Rapid Resolution HT Cartridge, 400 bar	2.1 x 15	1.8	921700-902	
RR	Rapid Resolution HT Cartridge, 3/pk, 400 bar	2.1 x 15	1.8	921700-932	
RR	Hardware Kit for RR and RRHT Cartridges			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	



Sterically Protected StableBond Bonded Phase

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, and 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 making StableBond columns 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	0.8-8.0	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

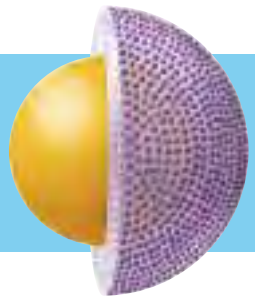
Specifications represent typical values only

*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 0.01-0.02 M. At mid-range pH, Eclipse Plus, Eclipse XDB and Bonus-RP are recommended.

TIPS & TOOLS

ZORBAX StableBond SB-C18, SB-C8 and SB-Aq phases are also available on Poroshell 120.

Turn to page 228



StableBond SB-C18 shows excellent stability at low pH and high temperature (pH 0.8, 90 °C)

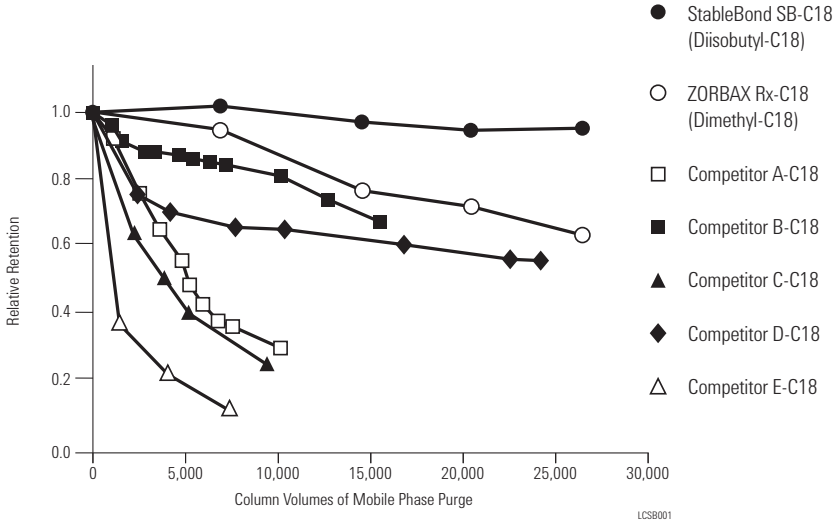
Column: **ZORBAX SB-C18**
883975-902
4.6 x 150 mm, 5 µm

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

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 150 mm, 5 µm

Mobile Phase: 0.1% TFA, pH 2:ACN

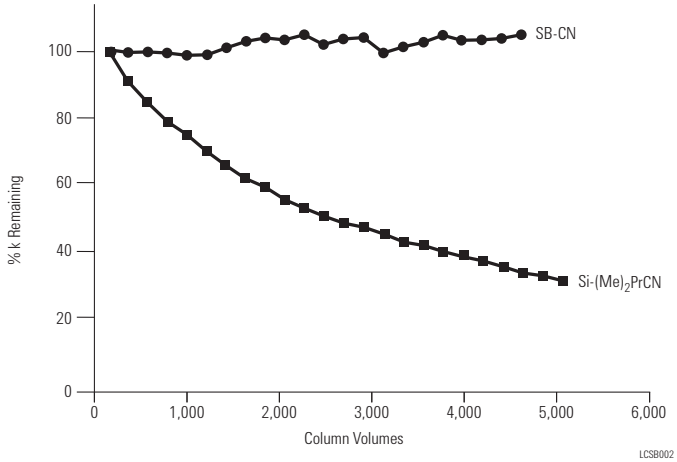
Flow Rate: 1 mL/min

Gradient: 0-100% ACN

Temperature: 50 °C

Sample: 1-phenylheptane @ 50% AC/50% water with 0.1% TFA

ZORBAX StableBond SB-CN and 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

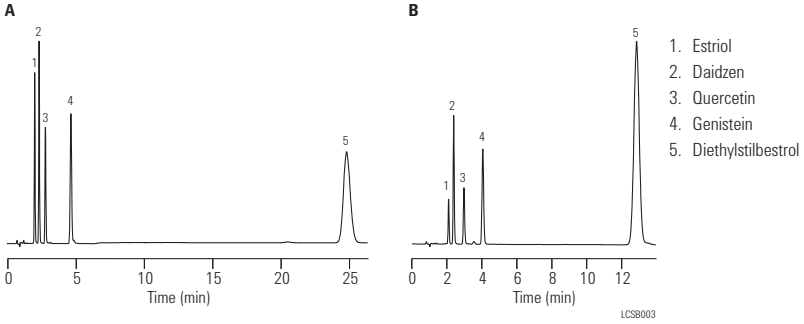
Column A: **ZORBAX SB-C18**
866953-902
4.6 x 75 mm, 3.5 µm

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

Mobile Phase: 30% ACN
70% 25mM NaH_2PO_4, 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.

Five different bonded phases provide selectivity options

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

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

Column C: **ZORBAX SB-C3**
883975-909
4.6 x 150 mm, 5 µm

Column D: **ZORBAX SB-Phenyl**
883975-912
4.6 x 150 mm, 5 µm

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

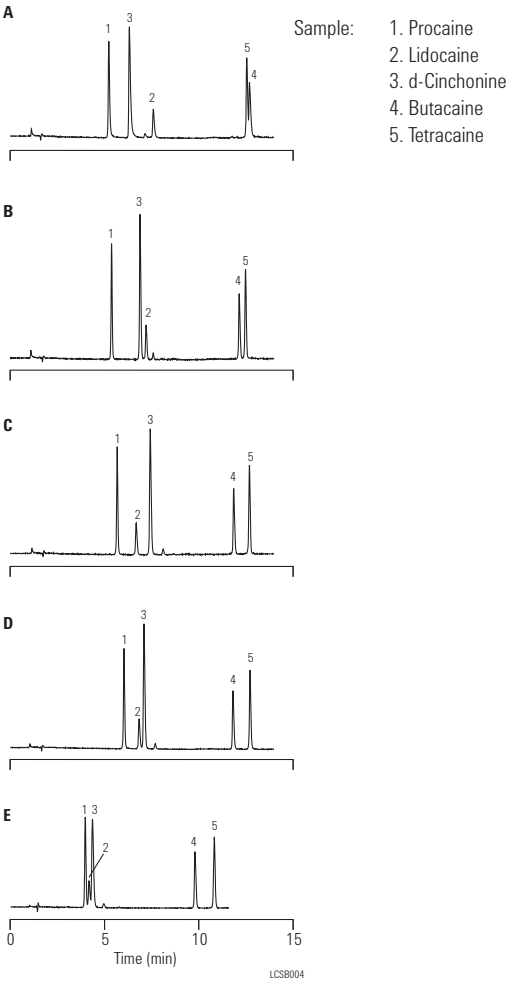
Mobile Phase: 0-100% B in 18.8 min
A: 50 mM NaH_2PO_4,
pH 2.5 in 95% H_2O / 5% ACN
B: 50 mM NaH_2PO_4,
pH 2.5 in 47% H_2O / 53% ACN

Flow Rate: 1.0 mL/min

Temperature: 26 °C

Detector: 254 nm

SB-C3 is just one of the five different StableBond selectivity choices. In this example, optimum resolution is obtained with SB-C3. All are based on the same high purity Rx-SIL. Selectivity changes are therefore dependent only on the bonded phases, making method development more reliable.



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)									
	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	4.6 x 30	3.5	834975-902	834975-906				
	Rapid Resolution	4.6 x 20	3.5	832975-902	832975-906				
	Rapid Resolution HT, 600 bar	4.6 x 150	1.8	829975-902	829975-906	829975-905		829975-912	829975-914
	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 75	1.8		830975-906				
	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				
UG	UHPLC Guard, 600 bar, 3/pk	4.6 x 5	1.8	820750-902	820750-904				
	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 Plus	3.0 x 75	3.5	866953-302					

Unless indicated, column pressure limit is 400 bar.

(Continued)

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)									
	Solvent Saver RRHD, 1200 bar	3.0 x 150	1.8	859700-302	859700-306				
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	858700-302	858700-306	858700-305		858700-312	
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	857700-302	857700-306	857700-305		857700-312	
	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	828975-309	828975-312	828975-314
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	827975-302	827975-306	827975-305			
	Solvent Saver HT, 600 bar	3.0 x 30	1.8	824975-302	824975-306	824975-305		827975-312	827975-314
	Solvent Saver HT, 600 bar	3.0 x 20	1.8	826975-302	826975-306				
UG	UHPLC Guard, 1200 bar, 3/pk	3.0 x 5	1.8	823750-902	823750-904				
	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
	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				
	Narrow Bore RRHD, 1200 bar	2.1 x 150	1.8	859700-902	859700-906	859700-905		859700-912	
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	858700-902	858700-906	858700-905		858700-912	
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	857700-902	857700-906	857700-905		857700-912	

Unless indicated, column pressure limit is 400 bar.

(Continued)

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)									
	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				
UG	UHPLC Guard, 1200 bar, 3/pk	2.1 x 5	1.8	821725-902	821725-904				
	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, 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	820675-124	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	0	840140-901	840140-901	840140-901	840140-901	840140-901	
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901	820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)									
PL	PrepHT Cartridge	21.2 x 250	7	877250-102	877250-106	877250-105		877250-112	877250-114
PL	PrepHT Cartridge	21.2 x 150	7	877150-102	877150-106				877150-114
PL	PrepHT Cartridge	21.2 x 150	5	870150-902	870150-906				870150-914
PL	PrepHT Cartridge	21.2 x 100	5	870100-902	870100-906				870100-914
PL	PrepHT Cartridge	21.2 x 50	5	870050-902	870050-906				870050-914
PL	PrepHT Guard Cartridge, 2/pk	17.0 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

Unless indicated, column pressure limit is 400 bar.

ZORBAX 80Å StableBond

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-Phenyl USP L11
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	
Standard Columns (no special hardware required)						
	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 Cartridges (require hardware kit 820555-901)						
RR	Rapid Resolution Cartridge	4.6 x 30	3.5	833975-902	833975-906	833975-912
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	
Rapid Resolution HT Cartridges (require hardware kit 820555-901)						
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		

ZORBAX 80Å StableBond

Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1
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

ZORBAX Rx

- Recommended for alternate selectivity at low pH relative to Eclipse Plus C18, Eclipse XDB-C18 and StableBond SB-C18; for higher temperature applications, StableBond is recommended
- Higher carbon load than SB-C18 columns (12% vs. 10%)
- High stability and good peak shape for low pH applications (up to pH 8)
- Manufactured using dimethyloctadecylsilane and non-endcapped
- ZORBAX 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	2.0-8.0	No	12%
ZORBAX Rx-C8	80Å	180 m^2/g	80 °C	1.0-8.0	No	5.5%

Specifications represent typical values only

*At pH 6-9 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 0.01-0.02 M.

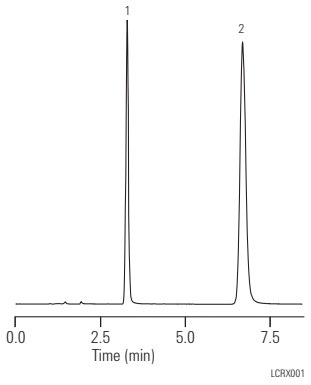
Analysis of diazepam on Rx-C18

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

Mobile Phase: 35% H_2O:65% MeOH

Flow Rate: 0.5 mL/min

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

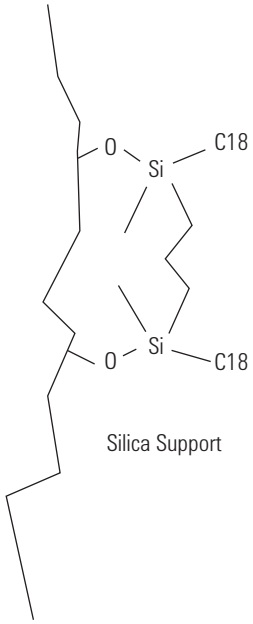


1. Ethylparaben
2. Diazepam

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	
	Rapid Resolution	4.6 x 100	3.5	861967-902	
	Rapid Resolution	4.6 x 75	3.5	866967-902	
	Solvent Saver	3.0 x 250	5	880967-302	
	Solvent Saver	3.0 x 150	5	883967-302	
	Solvent Saver Plus	3.0 x 150	3.5	863967-302	
	Solvent Saver Plus	3.0 x 100	3.5	861967-302	
	Narrow Bore	2.1 x 150	5	883700-902	
	Narrow Bore RR	2.1 x 100	3.5	861767-902	
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			820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)					
PHT	PrepHT Cartridge	21.2 x 250	7	877967-102	877250-106
PHT	PrepHT Cartridge	21.2 x 150	7		877150-106
PHT	PrepHT Cartridge	21.2 x 150	5		870150-906
PHT	PrepHT Cartridge	21.2 x 100	5		870100-906
PHT	PrepHT Cartridge	21.2 x 50	5		870050-906
PHT	PrepHT Guard Cartridge, 2/pk		5	820212-914	820212-915
PHT	Guard Cartridge Hardware			820444-901	820444-901
PHT	PrepHT Endfittings, 2/pk			820400-901	820400-901

*Rx-C8 is the same product as SB-C8. For other sizes and configurations, see the ZORBAX StableBond section, page 264.



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.

TIPS & TOOLS

Always use Agilent Certified Lamps for Best LC Performance

Agilent detector lamps are built to the tightest specifications and quality standards. They are designed to increase light intensity and decrease noise, which improves chromatographic results. Agilent rigorously tests its lamps for lowest lamp-to-lamp variability. Trust Agilent lamps for robust, long-lasting performance and lower cost of ownership. To learn more, visit **www.agilent.com/chem/lamps**

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%

Specifications represent typical values only.

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

**Above pH 6 highest column stability for all silica based columns is obtained by reducing the operating temperature to 40 °C or below and using lower buffer concentrations (0.01-0.02 M) or organic buffers.

Basic antihistamines on Extend-C18 at high pH

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

Mobile Phase: pH 7:
30% 20 mM Na_2HPO_4 70% MeOH
pH 11:
30% 20 mM TEA 70% MeOH

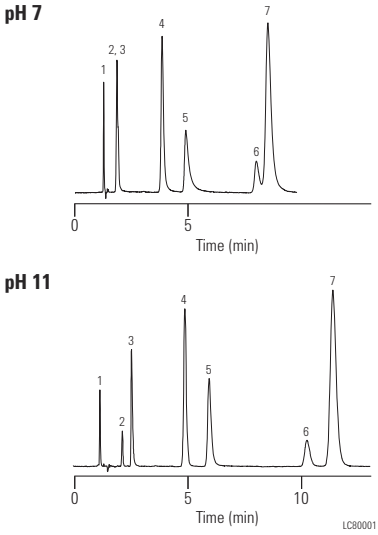
Flow Rate: 1.0 mL/min

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.



1. Maleate
2. Scopolamine
3. Pseudoephedrine
4. Doxylamine
5. Chlorpheniramine
6. Triprolidine
7. Diphenhydramine

Long life at high pH with Extend-C18

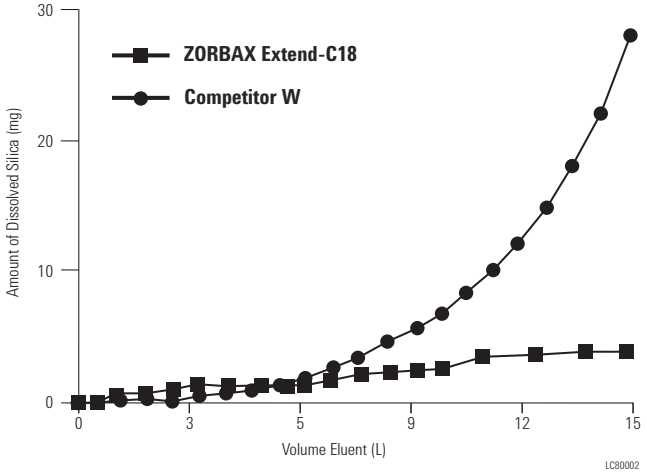
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

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 150 mm, 5 µm

Mobile Phase: 80% 25 mM NaH_2PO_4, pH 3.0
20% Methanol

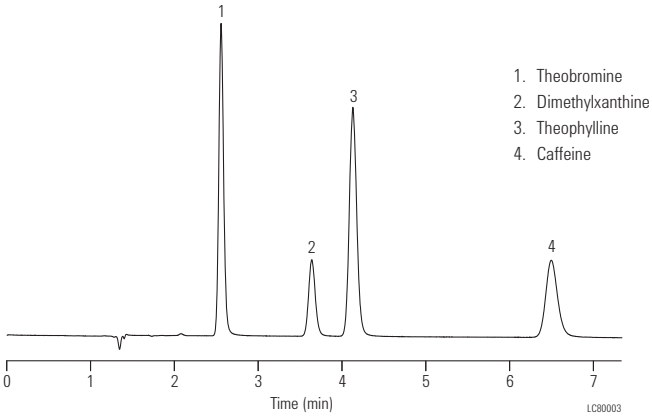
Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 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)	Extend-C18 USP L1
Standard Columns (no special hardware required)				
	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, 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

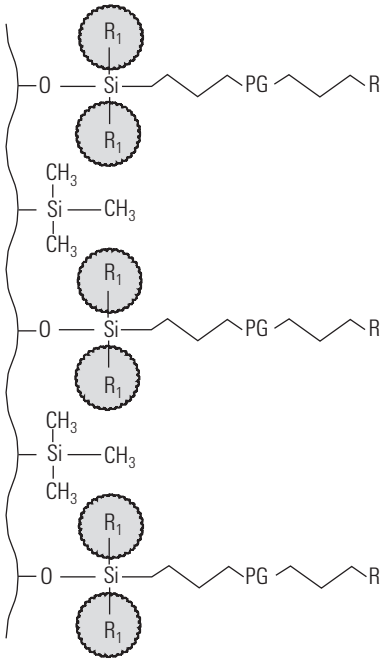
Unless indicated, column pressure limit is 400 bar.

(Continued)

ZORBAX 80Å Extend-C18

Hardware	Description	Size (mm)	Particle Size (µm)	Extend-C18 USP L1
Standard Columns (no special hardware required)				
	Solvent Saver RRHD, 1200 bar	3.0 x 100	1.8	758700-302
	Solvent Saver RRHD, 1200 bar	3.0 x 50	1.8	757700-302
	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
	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 RRHD, 1200 bar	2.1 x 150	1.8	759700-902
	Narrow Bore RRHD, 1200 bar	2.1 x 100	1.8	758700-902
	Narrow Bore RRHD, 1200 bar	2.1 x 50	1.8	757700-902
	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, 3/pk	1.0 x 17	5	5185-5923
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-930
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-930
ZGC	Guard Hardware Kit			820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)				
PL	PrepHT Cartridge	21.2 x 150	5	770150-902
PL	PrepHT	21.2 x 100	5	770100-902
PL	PrepHT	21.2 x 50	5	770050-902
PL	PrepHT Endfittings, 2/pk			820400-901
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-930
PL	Guard Cartridge Hardware			820444-901

Unless indicated, column pressure limit is 400 bar.



Unique, Polar Alkyl Bonus-RP Bonded Phase

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%

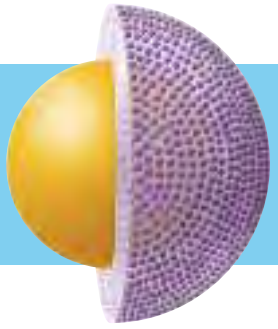
Specifications represent typical values only.

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

TIPS & TOOLS

ZORBAX Bonus-RP is also available on Poroshell 120.

Turn page 228



Improved peak shape of basic compounds using Bonus-RP

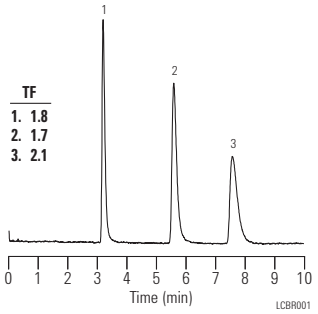
Column: **Alkyl-C8**
4.6 x 150 mm, 5 µm

Mobile Phase: 75% 25 mM NH_4OAc, pH 5.5
25% ACN

Flow Rate: 1.5 mL/min

Temperature: 40 °C

Detector: 254 nm



1. Doxylamine
2. Chlorpheniramine
3. Triprolidine

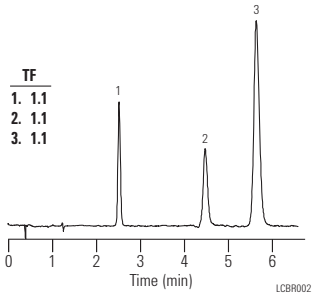
Column: **ZORBAX Bonus-RP**
883668-901
4.6 x 150 mm, 5 µm

Mobile Phase: 80% 25 mM NH_4OAc, pH 5.5
20% ACN

Flow Rate: 1.5 mL/min

Temperature: 40 °C

Detector: 254 nm



1. Doxylamine
2. Chlorpheniramine
3. Triprolidine

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.

ZORBAX Bonus-RP is stable at low and mid pH

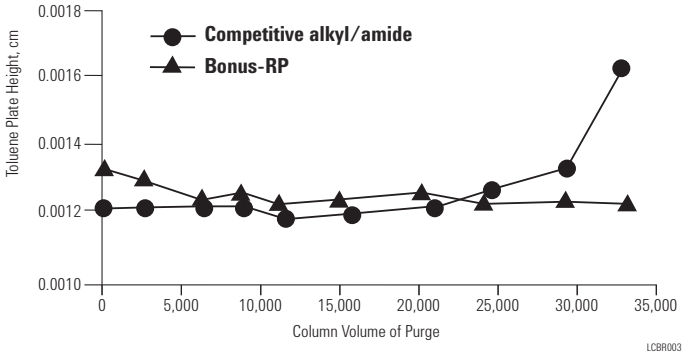
Column: **ZORBAX Bonus-RP**
883668-901
4.6 x 150 mm, 5 µm

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 volume is equivalent to approximately one working month.



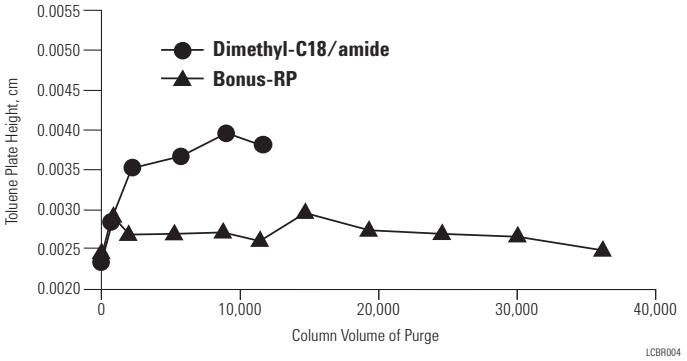
Dimethyl-C18/amide, Bonus-RP

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

Mobile Phase: Aging:
50% MeOH
50% 0.1% TFA
Test:
80% MeOH
20% H_2O

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 provides unique selectivity

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

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

Mobile Phase: 75% 25 mM Na Citrate, pH 6
25% MeOH

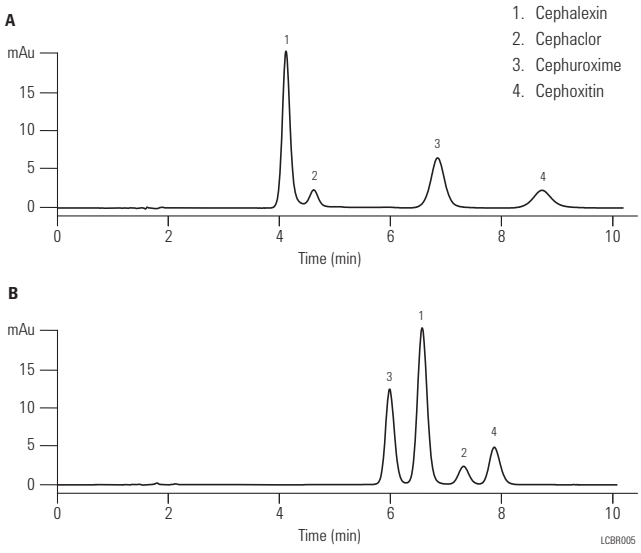
Flow Rate: 1.0 mL/min

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 changes.



ZORBAX Bonus-RP

Hardware	Description	Size (mm)	Particle Size (µm)	Bonus-RP USP L60
Standard Columns (no special hardware required)				
	Analytical	4.6 x 250	5	880668-901
	Analytical	4.6 x 150	5	883668-901
	Rapid Resolution	4.6 x 250	3.5	884950-577
	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
	Rapid Resolution	4.6 x 50	3.5	835668-901
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	828668-901
	Rapid Resolution HT, 600 bar	4.6 x 75	1.8	830668-901
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	827668-901
	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
	Solvent Saver HT, 600 bar	3.0 x 100	1.8	828668-301
	Solvent Saver HT, 600 bar	3.0 x 50	1.8	827668-301
	Rapid Resolution HD, 1200 bar	2.1 x 150	1.8	859768-901
	Rapid Resolution HD, 1200 bar	2.1 x 100	1.8	858768-901
	Rapid Resolution HD, 1200 bar	2.1 x 50	1.8	857768-901
	Narrow Bore	2.1 x 150	5	883725-901
	Narrow Bore	2.1 x 50	5	861971-901

Unless indicated, column pressure limit is 400 bar.

(Continued)

ZORBAX Bonus-RP bonding is also available on Poroshell 120 columns. Turn to page 228.

ZORBAX Bonus-RP

Hardware	Description	Size (mm)	Particle Size (µm)	Bonus-RP USP L60
Standard Columns (no special hardware required)				
	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
	Narrow Bore RRHT, 600 bar	2.1 x 100	1.8	828768-901
	Narrow Bore RRHT, 600 bar	2.1 x 50	1.8	827768-901
	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, 3/pk	1.0 x 17	5	5185-5922
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-928
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-928
ZGC	Guard Hardware Kit			820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)				
PH	PrepHT Cartridge	21.2 x 250	7	878250-101
PH	PrepHT Cartridge	21.2 x 150	7	878150-101
PH	PrepHT Cartridge	21.2 x 150	5	868150-901
PH	PrepHT Cartridge	21.2 x 100	5	868100-901
PH	PrepHT Cartridge	21.2 x 50	5	868050-901
PH	PrepHT Endfittings, 2/pk			820400-901
PH	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-928
PH	Guard Cartridge Hardware			820444-901

Unless indicated, column pressure limit is 400 bar.

TIPS & TOOLS

Watch LC troubleshooting videos featuring Agilent chromatographic experts at **www.agilent.com/chem/lctroubleshooting**

ZORBAX Original Reversed-Phase Columns

Agilent 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.

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp Limit	pH Range	Endcapped	Carbon Load
ZORBAX C18	70Å	300 m^2/g	60 °C	2.0 - 8.0	Yes/No	20%
ZORBAX C8	70Å	300 m^2/g	60 °C	2.0 - 8.0	Yes	12%
ZORBAX-Phenyl	70Å	300 m^2/g	60 °C	2.0 - 8.0	Yes	12%
ZORBAX CN	70Å	300 m^2/g	60 °C	2.0 - 8.0	N/A	7%
ZORBAX-TMS	70Å	300 m^2/g	60 °C	2.0 - 7.0	N/A	4%

ZORBAX Original Reversed-Phase Columns

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)								
	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			840140-901	840140-901	840140-901	840140-901	840140-901
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)								
PHT	PrepHT Cartridge	21.2 x 250	7	877952-102	877952-106		877952-105	
PHT	PrepHT Endfittings, 2/pk			820400-901	820400-901		820400-901	

ZORBAX Method Development Kits

Kits for Analytical HPLC

ZORBAX Method Development Kits

Agilent offers a series of kits that allow for fast method development at an attractive price. Each kit contains 3 columns. Six new kits have been added and are recommended for use with the new Agilent Automated Method Development LC. Several of these kits contain Rapid Resolution HT (1.8 µm) columns in a variety of bonded phases for easy method optimization and several kits contain Rapid Resolution (3.5 µm) columns in the same variety of bonded phases. These kits contain some of the Eclipse Plus family of columns for excellent peak shape and optimum performance with a wide variety of compounds.

ZORBAX Method Development Kits
Recommended for use with the Agilent Automated Method Development LC System

Description	Part No.
Rapid Resolution HT (RRHT) Selectivity Method Development Kit, 2.1 mm id Includes 2.1 x 50 mm, 1.8 µm, 600 bar columns: one each Eclipse Plus C18, Eclipse Plus Phenyl-Hexyl and Bonus-RP	5190-1431
Rapid Resolution HT (RRHT) pH Method Development Kit, 2.1 mm id Includes 2.1 x 50 mm, 1.8 µm, 600 bar columns: one each Eclipse Plus C18, SB-C18 and Extend-C18	5190-1432
Rapid Resolution HT (RRHT) Selectivity Method Development Kit, 4.6 mm id Includes 4.6 x 50 mm, 1.8 µm, 600 bar columns: one each Eclipse Plus C18, Eclipse Plus Phenyl-Hexyl and Bonus-RP	5190-1433
Rapid Resolution HT (RRHT) pH Method Development Kit, 4.6 mm id Includes 4.6 x 50 mm, 1.8 µm, 600 bar columns: one each Eclipse Plus C18, SB-C18 and Extend-C18	5190-1434
Rapid Resolution Selectivity Method Development Kit, 4.6 mm id Includes 4.6 x 100 mm, 3.5 µm columns: one each Eclipse Plus C18, Eclipse Plus Phenyl-Hexyl and Bonus-RP	5190-1435
Rapid Resolution pH Method Development Kit, 4.6 mm id Includes 4.6 x 100 mm, 3.5 µm columns: one each Eclipse Plus C18, SB-C18 and Extend-C18	5190-1436

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 Cartridge Column Starter Kits

Hardware	Description	Part No.
AC	ZORBAX C18 Kit Includes one 4.6 x 150 mm, 5 µm Eclipse XDB-C18 column; one 4.6 x 150 mm, 5 µm StableBond C18 column; cartridge holder; mounting tool; replacement filter (2/pk); and open-end wrench	5183-2021
AC	ZORBAX C8 Kit Includes one 4.6 x 150 mm, 5 µm Eclipse XDB-C8 column; one 4.6 x 150 mm, 5 µm StableBond C8 column; cartridge holder; mounting tool; replacement filter (2/pk); and open-end wrench	5183-2022

ZORBAX Method Validation Kits

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 Agilent Authorized Distributor using the following procedure:

- Request Validation Kits (columns from different lots) by using Part Number 899999-888
- Indicate the Part Number of the current column you are using
- Indicate the Lot Number of the current column you are using
- Indicate the number of additional columns needed from different lots (example: you have a current column and may need two additional lots)
- Please fax your request to **(302) 993-5354** (United States and Canada) or email to **cag_sales-na@agilent.com**. You will receive a quote from your Customer Service Representative within 1-2 business days. Delivery of your method validation kit is usually 3 weeks or less from the time your order is placed, depending on lot availability.

Custom HPLC Column Ordering

Columns not listed can be easily ordered using the following procedure:

- Request a Special Products Quotation (SPQ) using Part Number 899999-999
- Indicate column dimensions (example: 4.6 x 50 mm); bonded phase type (example: StableBond C3); particle size (example: 5 µm); and pore size (example: 80Å)
- Please fax your request to **(302) 993-5354** (United States and Canada) or email to **cag_sales-na@agilent.com**. You will receive a quote from your Customer Service Representative within 1-2 business days. Delivery of your custom column is usually 3 weeks or less from the time your order is placed, depending on lot availability.

Custom columns are priced with a minimal surcharge over the price of stocked columns.

Pursuit HPLC Columns

Beginning in drug discovery and drug metabolism, Pursuit columns are ideal for analyzing lead compounds and biological samples. The column's performance is due to the unique combination of advanced bonding chemistry and ultra-high purity silica. These factors combine to provide rapid separations with excellent first time resolution and symmetrical peaks for polar compounds, whether at pH 1.5 or 10. Additionally, the need for ion-pairing agents such as TFA is often eliminated, thus maximizing the performance of single and parallel multi-channel LC/MS systems.

Culminating in QC, Pursuit is ideal for implementing dependable trouble-free analysis of raw materials and approved drugs. Rigorous control and validation of each step in the manufacturing process ensures column reproducibility. With Pursuit, your laboratory can spend its energy on producing results.

Special columns, such as Pursuit PFP (for very polar compounds) and Pursuit PAH (environmental), give you the extra selectivities you need for your most challenging applications.

Pursuit HPLC Columns

Pursuit

For LC/MS and high throughput applications, the Pursuit column is built on the larger 200Å pore size silica. High ligand density delivers up to 40% faster separations without sacrificing resolution. This is accomplished by optimizing mass transfer with the larger pore size.

Pursuit XRs

Pursuit XRs columns are for performance in analytical R&D, QC and preparative applications. Combining high ligand density with a 100Å pore size, high surface area silica, Pursuit XRs columns are designed to increase productivity, as they offer maximum loadability, excellent stability and easy scalability while maintaining superior resolution.

Pursuit XRs Ultra

For the ultimate in speed and good resolution on any instrument, we designed the Pursuit XRs Ultra around an optimized 2.8 µm particle and an advanced packing procedure. Now you can decrease your run time while maintaining resolution. Lower backpressure allows high flow rates to be used, and the 2.8 µm particles of ultra-pure silica delivers 10-15% higher efficiency than 3 µm columns.

Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Endcapped	Carbon Load	Pore Volume	Ligand Coverage
Pursuit C18	200Å	200 m^2/g	1.5-10	Yes	12.9%	11 mL/g	3.5 $\mu mol/m^2$
Pursuit C8	200Å	200 m^2/g	1.5-10	Yes	7.4%	11 mL/g	3.8 $\mu mol/m^2$
Pursuit Diphenyl	200Å	200 m^2/g	1.5-8.0	Yes	7.3%	11 mL/g	2.8 $\mu mol/m^2$
Pursuit PFP	200Å	200 m^2/g	1.5-10	Yes	6.3%	11 mL/g	3.4 $\mu mol/m^2$
Pursuit PAH	200Å	200 m^2/g	1.5-10	Yes		11 mL/g	
Pursuit XRs C18	100Å	440 m^2/g	1.5-10	Yes	22%	11 mL/g	2.9 $\mu mol/m^2$
Pursuit XRs C8	100Å	440 m^2/g	1.5-10	Yes	15%	11 mL/g	3.7 $\mu mol/m^2$
Pursuit XRs Diphenyl	100Å	440 m^2/g	1.5-8.0	Yes	14.6%	11 mL/g	2.6 $\mu mol/m^2$
Pursuit XRs Si	100Å	440 m^2/g	1.5-10	Yes		11 mL/g	
Pursuit XRs Ultra C18	100Å	440 m^2/g	1.5-10	Yes	23.2%	11 mL/g	3.2 $\mu mol/m^2$
Pursuit XRs Ultra C8	100Å	440 m^2/g	1.5-10	Yes	15%	11 mL/g	3.7 $\mu mol/m^2$
Pursuit XRs Ultra Diphenyl	100Å	440 m^2/g	1.5-8.0	Yes	14.6%	11 mL/g	2.6 $\mu mol/m^2$

Specifications represent typical values only

TIPS & TOOLS

Request custom LC columns online at **www.agilent.com/chem/customlccol**

Tricyclic antidepressants and benzodiazepines

Column: **Pursuit XRs C18**
A6000150X046
4.6 x 150 mm, 5 µm

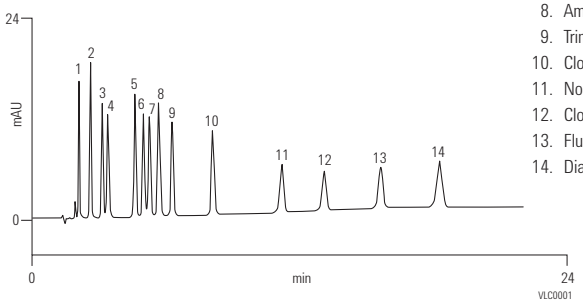
Mobile Phase: A: Water+0.1% HCOOH
B: MeCN+0.1% HCOOH

Gradient: 30-40% B in 15 min, hold at 40% B for 15 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. 7-Aminoclonazepam
2. 7-Aminoflunitrazepam
3. Nordoxepin
4. Doxepin
5. Desipramine
6. Imipramine
7. Nortriptyline
8. Amitriptyline
9. Trimipramine
10. Clomipramine
11. Nordiazepam
12. Clonazepam
13. Flunitrazepam
14. Diazepam

Mechanical stability of Pursuit XRs

Column: **Pursuit XRs C18**
A6000050X020
2.0 x 50 mm, 5 µm

Sample: DMSO mix

Mobile Phase: A: MeOH:water, 10:90 + 0.1% HCOOH
B: MeOH:water, 90:10 + 0.1% HCOOH

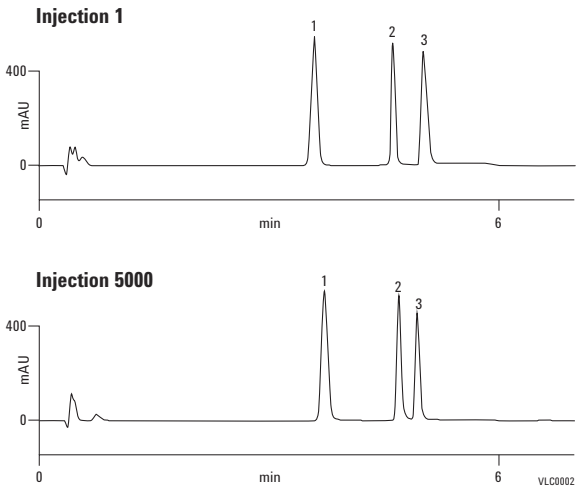
Gradient: 0-100% B in 3 min, back to 0% B in 0.5 min, hold at 0% B for 3.5 min

Flow Rate: 0.4 mL/min

Temperature: Ambient

Detector: UV, 254 nm

1. 4-Methoxybenzenesulfonamide
2. Methyl 3-aminothiophene-2-carboxylate
3. Trimipramine



Antifungals

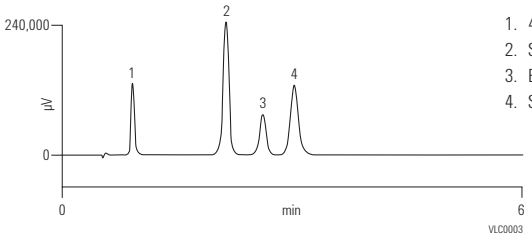
Column: **Pursuit XRs Ultra Diphenyl**
A7521050X020
2.0 x 50 mm, 2.8 µm

Mobile Phase: Water+0.1% HCOOH:MeCN+0.1% HCOOH, 80:20

Flow Rate: 0.4 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. 4-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid
4. Salicylic acid

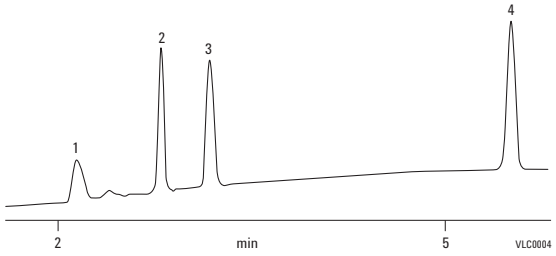
Liquid chromatography phase test mixture (LPTM) on Pursuit C8

Column: **Pursuit C8**
A3031050X020
2.0 x 50 mm, 3 µm

Mobile Phase: A: 0.05% HCOOH in water
B: 0.05% HCOOH in MeCN

Flow Rate: 0.6 mL/min

Detector: UV, 220 nm



1. Aspartame
2. Cortisone
3. Reserpine
4. Dioctyl phthalate

Adrenocorticosteroids on Pursuit PFP and C18

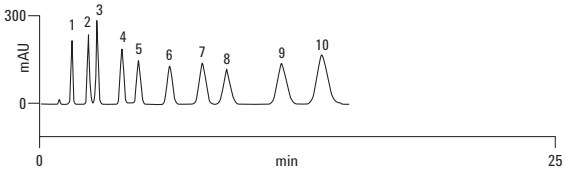
Mobile Phase: MeCN:water, 22.5:77.5

Flow Rate: 1.5 mL/min

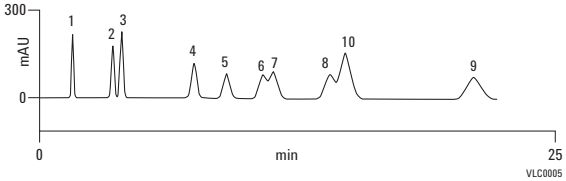
Temperature: Ambient

Detector: UV, 240 nm

Pursuit PFP



Pursuit C18



1. Triamcinolone
2. Prednisolone
3. Cortisone
4. Methylprednisolone
5. Corticosterone
6. Beclomethasone
7. Prednisolone acetate
8. Triamcinolone acetonide
9. Cortisone acetate
10. Fluocinolone acetonide

Pursuit HPLC Columns

Semi-Prep Scale

Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit Diphenyl	Pursuit PFP	Pursuit PAH USP L1
10.0 x 250	10	A3002250X100	A3032250X100			
10.0 x 150	5	A3000150X100			A3050150X100	
10.0 x 250	5	A3000250X100	A3030250X100		A3050250X100	

Pursuit HPLC Columns

Analytical Scale

Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit Diphenyl	Pursuit PFP	Pursuit PAH USP L1
4.6 x 250	10	A3002250X046	A3032250X046			
4.6 x 150	10	A3002150X046	A3032150X046			
4.6 x 100	10	A3002100X046	A3032100X046			
4.6 x 250	5	A3000250X046	A3030250X046	A3040250X046	A3050250X046	A7000250X046
4.6 x 150	5	A3000150X046	A3030150X046	A3040150X046	A3050150X046	A7000150X046
4.6 x 100	5	A3000100X046	A3030100X046	A3040100X046	A3050100X046	
4.6 x 50	5	A3000050X046	A3030150X046	A3040050X046	A3050050X046	
4.6 x 250	3	A3001250X046	A3031250X046	A3041250X046	A3051250X046	
4.6 x 150	3	A3001150X046	A3031150X046	A3041150X046	A3051150X046	
4.6 x 100	3	A3001100X046	A3031100X046	A3041100X046	A3051100X046	A7001100X046
4.6 x 50	3	A3001050X046		A3041050X046	A3051050X046	
4.6 x 30	3	A3001030X046				
4.0 x 250	5	A3000250X040				
4.0 x 125	5	A3000125X040				
3.9 x 300	10	A3002300X039				
3.9 x 300	5	A3000300X039				
3.9 x 150	5	A3000150X039				
3.0 x 250	5	A3000250X030		A3040250X030		
3.0 x 150	5	A3000150X030		A3040150X030	A3050150X030	
3.0 x 100	5	A3000100X030			A3050100X030	
3.0 x 250	3	A3001250X030				
3.0 x 150	3	A3001150X030		A3041150X030	A3051150X030	
3.0 x 100	3	A3001100X030		A3041100X030	A3051100X030	A7001100X030

(Continued)

Pursuit HPLC Columns

Analytical Scale

Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit Diphenyl	Pursuit PFP	Pursuit PAH USP L1
3.0 x 50	3	A3001050X030		A3041050X030	A3051050X030	
2.0 x 250	5	A3000250X020				
2.0 x 150	5	A3000150X020	A3030150X020	A3040150X020		
2.0 x 100	5	A3000100X020	A3030100X020	A3040100X020	A3050100X020	
2.0 x 50	5	A3000050X020	A3030050X020	A3040050X020	A3050050X020	
2.0 x 30	5	A3000030X020		A3040030X020	A3050030X020	
2.0 x 20	5	A3000020X020			A3050020X020	
2.0 x 250	3	A3001250X020		A3041250X020		
2.0 x 200	3			A3041200X020		
2.0 x 150	3	A3001150X020	A3031150X020	A3041150X020	A3051150X020	
2.0 x 100	3	A3001100X020	A3031100X020	A3041100X020	A3051100X020	A7001100X020
2.0 x 50	3	A3001050X020	A3031050X020	A3041050X020	A3051050X020	
2.0 x 30	3	A3001030X020	A3031030X020	A3041030X020	A3051030X020	
2.0 x 20	3	A3001020X020		A3041020X020	A3051020X020	

Pursuit HPLC Columns

Prep Scale

Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit Diphenyl	Pursuit PFP	Pursuit PAH USP L1
50.0 x 250	10	A3002250X500	A3032250X500			
21.2 x 250	10	A3002250X212	A3032250X212			
21.2 x 150	10	A3002150X212				
21.2 x 250	5	A3000250X212			A3050250X212	
21.2 x 150	5	A3000150X212			A3050150X212	
21.2 x 100	5			A3040100X212		

Pursuit ChromSep Complete Cartridge Systems

Hardware	Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit PAH USP L1
CS	4.6 x 250	5	A3000250C046	A3030250C046	A7000250C046
CS	4.6 x 250	3		A3031250C046	
CS	4.6 x 150	5	A3000150C046	A3030150C046	A7000150C046
CS	4.6 x 100	5	A3000100C046	A3030100C046	
CS	4.6 x 150	3	A3001150C046	A3031150C046	A7001150C046
CS	4.6 x 100	3	A3001100C046	A3031100C046	A7001100C046
CS	4.6 x 50	3	A3001050C046		
CS	3.0 x 250	5	A3000250C030		
CS	3.0 x 150	5	A3000150C030		
CS	3.0 x 100	5	A3000100C030		A7000100C030
CS	3.0 x 150	3	A3001150C030		
CS	3.0 x 100	3	A3001100C030		
CS	2.0 x 250	5	A3000250C020		
CS	2.0 x 150	5	A3000150C020	A3030150C020	
CS	2.0 x 100	5	A3000100C020		
CS	2.0 x 150	3	A3001150C020		
CS	2.0 x 100	3	A3001100C020		
CS	2.0 x 50	3	A3001050C020		

Pursuit ChromSep Replacement Cartridges

Hardware	Size (mm)	Particle Size (µm)	Unit	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit PAH USP L1
CS	4.6 x 250	5				A7000250R046
			3/pk			A7000250T046
CS	4.6 x 150	5		A3000150R046	A3030150R046	A7000150R046
			3/pk	A3000150T046	A3030150T046	A7000150T046
CS	4.6 x 150	3			A3031150R046	A7001150R046
			3/pk		A3031150T046	A7001150T046
CS	4.6 x 100	3				A7001100R046
			3/pk			A7001100T046
CS	4.6 x 50	3		A3001050R046		
			3/pk	A3001050T046		
CS	3.0 x 150	5		A3000150R030		
			3/pk	A3000150T030		
CS	3.0 x 100	5		A3000100R030		A7000100R030
			3/pk	A3000100T030		A7000100T030
CS	3.0 x 150	3		A3001150R030		
			3/pk	A3001150T030		
CS	3.0 x 100	3		A3001100R030		A7001100R030
			3/pk	A3001100T030		A7001100T030
CS	2.0 x 50	3			A3031050R020	
			3/pk		A3031050T020	

MetaGuard Columns, 3/pk

Hardware	ID (mm)	Particle Size (µm)	Pursuit C18	Pursuit C8	Pursuit DP	Pursuit PFP
MG	4.6	10	A3002MG			
MG	2.0	10	A3002MG2			
MG	4.6	5	A3000MG	A3030MG	A3040MG	A3050MG
MG	2.0	5	A3000MG2	A3030MG2	A3040MG2	A3050MG2
MG	1.0	5	A3000MG1		A3040MG1	
MG	4.6	3	A3001MG	A3031MG	A3041MG	A3051MG
MG	2.0	3	A3001MG2	A3031MG2	A3041MG2	A3051MG2
MG	1.0	3			A3041MG1	

Pursuit XRs HPLC Columns

Semi-Prep Scale

Size (mm)	Particle Size (µm)	Pursuit XRs C18 USP L1	Pursuit XRs C8 USP L7	Pursuit XRs Diphenyl USP L11	Pursuit XRs Si* USP L3
10.0 x 250	10	A6002250X100			A6004250X100
10.0 x 250	5	A6000250X100		A6020250X100	
10.0 x 150	5	A6000150X100			
10.0 x 50	5	A6000050X100			
10.0 x 150	3			A6021150X100	

*Pursuit XRs Si is a normal phase column.

Pursuit XRs HPLC Columns

Analytical Scale

Size (mm)	Particle Size (µm)	Pursuit XRs C18 USP L1	Pursuit XRs C8 USP L7	Pursuit XRs Diphenyl USP L11	Pursuit XRs Si* USP L3
4.6 x 250	10	A6002250X046			A6004250X046
4.6 x 50	10	A6002050X046S			
4.6 x 250	5	A6000250X046	A6010250X046	A6020250X046	
4.6 x 150	5	A6000150X046	A6010150X046	A6020150X046	
4.6 x 100	5	A6000100X046	A6010100X046	A6020100X046	A6006100X046
4.6 x 50	5	A6000050X046		A6020050X046	A6006050X046
4.6 x 250	3	A6001250X046		A6021250X046	
4.6 x 150	3	A6001150X046	A6010150X046	A6021150X046	
4.6 x 100	3	A6001100X046	A6011100X046	A6021100X046	A6005100X046
4.6 x 50	3	A6001050X046	A6011050X046	A6021050X046	A6005050X046
4.6 x 30	3	A6001030X046		A6021030X046	
4.0 x 250	5	A6000250X040	A6010250X040		
4.0 x 150	5	A6000150X040	A6010150X040		
3.0 x 250	5	A6000250X030	A6010250X030	A6020250X030	
3.0 x 150	5	A6000150X030	A6010150X030	A6020150X030	
3.0 x 100	5	A6000100X030	A6010100X030	A6020100X030	
3.0 x 150	3	A6001150X030	A6011150X030	A6021150X030	
3.0 x 100	3	A6001100X030	A6011100X030	A6021100X030	
3.0 x 50	3	A6001050X030	A6011050X030	A6021050X030	
3.0 x 30	3	A6001030X030			

*Pursuit XRs Si is a normal phase column.

(Continued)

Pursuit XRs HPLC Columns

Analytical Scale

Size (mm)	Particle Size (µm)	Pursuit XRs C18 USP L1	Pursuit XRs C8 USP L7	Pursuit XRs Diphenyl USP L11	Pursuit XRs Si* USP L3
2.1 x 100	5				A6006100X021
2.0 x 250	5	A6000250X020		A6020250X020	
2.0 x 150	5	A6000150X020	A6010150X020	A6020150X020	
2.0 x 100	5	A6000100X020	A6010100X020		
2.0 x 50	5	A6000050X020	A6010050X020	A6020050X020	
2.0 x 30	5	A6000030X020			
2.0 x 250	3	A6001250X020		A6021250X020	
2.0 x 150	3	A6001150X020	A6011150X020	A6021150X020	
2.0 x 100	3	A6001100X020	A6011100X020	A6021100X020	
2.0 x 50	3	A6001050X020	A6011050X020	A6021050X020	A6005050X020
2.0 x 30	3			A6021030X020	
2.0 x 20	3	A6001020X020			
1.0 x 150	3	A6001150X010			
1.0 x 100	3	A6001100X010		A6021100X010	

*Pursuit XRs Si is a normal phase column.

Pursuit XRs HPLC Columns

Prep Scale

Size (mm)	Particle Size (µm)	Pursuit XRs C18 USP L1	Pursuit XRs C8 USP L7	Pursuit XRs Diphenyl USP L11	Pursuit XRs Si* USP L3
50.0 x 250	10	A6002250X500		A6002250X500	A6004250X500
30.0 x 250	5	A6000250X300			A6004250X300
30.0 x 150	5	A6000150X300		A6020150X300	
30.0 x 100	5	A6000100X300			
30.0 x 50	5	A6000050X300			
21.2 x 250	10	A6002250X212	A6012250X212		A6004250X212
21.2 x 250	5	A6000250X212		A6020250X212	
21.2 x 150	5	A6000150X212			
21.2 x 100	5	A6000100X212		A6020100X212	
21.2 x 50	5	A6000050X212			
21.2 x 30	5	A6000030X212			

*Pursuit XRs Si is a normal phase column.

MetaGuard Columns, 3/pk

Hardware	ID (mm)	Particle Size (µm)	Pursuit XRs C18	Pursuit XRs Si	Pursuit XRs C8	Pursuit XRs Diphenyl	Pursuit PAH
MG	4.6	10	A6002MG	A6004MG			
MG	4.6	5	A6000MG		A6010MG	A6020MG	
MG	3.0	5					A7000MG3
MG	2.0	5	A6000MG2		A6010MG2	A6020MG2	
MG	4.6	3	A6001MG		A6011MG	A6021MG	
MG	3.0	3					A7001MG3
MG	2.0	3	A6001MG2		A6011MG2	A6021MG2	A6001MG2

Pursuit XRs Ultra HPLC Columns

Size (mm)	Particle Size (µm)	Pursuit XRs Ultra C18	Pursuit XRs Ultra C8	Pursuit XRs Ultra Diphenyl
3.0 x 150	2.8	A7501150X030	A7511150X030	
3.0 x 100	2.8	A7501100X030		
2.0 x 150	2.8	A7501150X020		
2.0 x 100	2.8	A7501100X020	A7511100X020	A7521100X020
2.0 x 50	2.8	A7501050X020	A7511050X020	A7521050X020
2.0 x 30	2.8	A7501030X020	A7511030X020	A7521030X020

Polaris HPLC Columns

Polaris HPLC Columns

In areas like drug discovery where target compounds are increasingly polar, it is critical to have a reversed-phase column that performs well under aqueous conditions. Retention is critical, but cannot come with troublesome secondary interactions. Likewise, phase collapse and shifting retention times need to be avoided. The answer is our Polaris line of polar-modified columns.

From the collapse-resistant pore structure of our base silica, to the "wettability" engineered into the bonded phases, Polaris columns have been designed for high aqueous conditions. The combination of high phase density bonding, ultra pure silica, and silanol shielding leads to excellent peak shape among polar-modified columns.

As a family, Polaris offers a variety of polar modifications in both C18 and C8 chemistries.

Polaris C18-A

Polaris C18-A is the best starting place for separations where the benefits of polar-modified columns are desired. The polar modifications of C18-A help it avoid poor peak shape and retention issues in low organic conditions.

Polaris C8-A

Polaris C8-A offers an alternative selectivity to standard C8 phases and has a lower hydrophobicity than Polaris C18-A, making it ideal for polar samples, or faster overall analysis times.

Polaris C18-Ether

Polaris C18-Ether offers an alternative selectivity to Polaris C18-A and standard C18 phases, and typically delivers increased retention of polar compounds away from the void volume.

Polaris C8-Ether

Polaris C8-Ether offers an alternative selectivity to Polaris C8-A with particular utility for hydrogen bonding compounds.

Column Specifications

Bonded Phase	Pore Size	Surface Area	Carbon Load	Endcapped	Pore Volume	Ligand Coverage
Polaris C18-A	180Å	200 m^2/g	13.8%	Yes	1.1 cm^3/g	3.9 µmol/m^2
Polaris C8-A	180Å	200 m^2/g	7.4%	Yes	1.1 cm^3/g	4.8 µmol/m^2
Polaris C18-Ether	180Å	200 m^2/g	12.1%	Yes	1.1 cm^3/g	3.3 µmol/m^2
Polaris C8-Ether	180Å	200 m^2/g	7.1%	Yes	1.1 cm^3/g	4.5 µmol/m^2
Polaris Amide C18	180Å	200 m^2/g	15%	Yes	1.1 cm^3/g	4.4 µmol/m^2
Polaris NH2	180Å	200 m^2/g	5.5%	Amide	1.1 cm^3/g	3.8 µmol/m^2
Polaris Si-A	180Å	200 m^2/g	N/A	N/A	1.1 cm^3/g	N/A

Specifications represent typical values only.

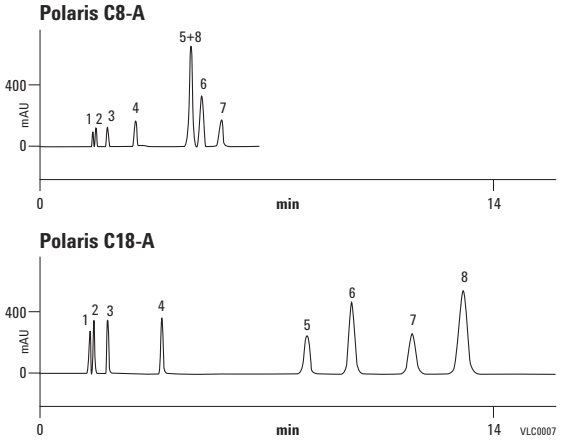
Selectivity test mix for Polaris columns

Mobile Phase: MeCN:water 70:30

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. Uracil
2. Caffeine
3. Phenol
4. Toluene
5. Butylbenzene
6. o-Terphenyl
7. Amylbenzene
8. Triphenylene

LC/MS performance test mix for Polaris C8-A

Column: Polaris C8-A
A2011030X030
3.0 x 30 mm, 3 µm

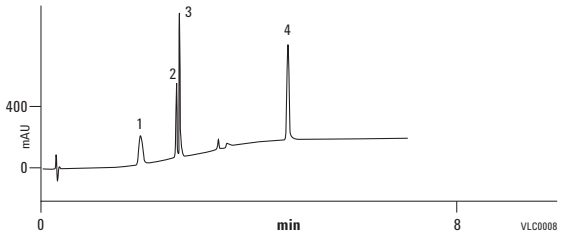
Mobile Phase: A: Water+0.05% HCOOH
B: MeCN+0.05% HCOOH

Gradient: 5-90% B in 3 min and hold for 4 min

Flow Rate: 0.6 mL/min

Temperature: Ambient

Detector: UV, 220 nm



1. Aspartame
2. Cortisone
3. Reserpine
4. Dioctyl phthalate

Polaris HPLC Columns

Size (mm)	Particle Size (µm)	Polaris C18-A	Polaris C8-A	Polaris C18-Ether	Polaris C8-Ether	Polaris Amide C18	Polaris NH2*	Polaris Si-A*
50.0 x 250	10	A2002250X500						A2004250X500
30.0 x 100	5	A2000100X300						
30.0 x 3.0	3					A2007030X030		
21.2 x 250	10	A2002250X212				A2008250X212		A2004250X212
21.2 x 250	5	A2000250X212	A2010250X212	A2020250X212	A2030250X212	A2006250X212	A2013250X212	A2003250X212
21.2 x 150	5	A2000150X212						A2003150X046
21.2 x 100	5	A2000100X212						
21.2 x 50	5							A2003050X212
10.0 x 250	10					A2008250X100		
10.0 x 250	5	A2000250X100		A2020250X100	A2030250X100	A2006250X100	A2013250X100	
10.0 x 50	3			A2021050X100				
4.6 x 250	10	A2002250X046						A2003250X046
4.6 x 250	5	A2000250X046	A2010250X046	A2020250X046	A2030250X046	A2006250X046	A2013250X046	
4.6 x 200	5	A2000200X046						
4.6 x 150	5	A2000150X046	A2010150X046	A2020150X046	A2030150X046	A2006150X046	A2013150X046	A2003150X046
4.6 x 100	5	A2000100X046	A2010100X046			A2006100X046	A2013100X046	A2003100X046
4.6 x 50	5	A2000050X046		A2020050X046		A2006050X046	A2013050X046	A2003050X046
4.6 x 30	5	A2000030X046						
4.6 x 250	3	A2001250X046		A2021250X046	A2031250X046	A2007250X046	A2014250X046	A2005250X046
4.6 x 150	3	A2001150X046	A2011150X046			A2007150X046	A2014150X046	A2005150X046
4.6 x 100	3	A2001100X046	A2011100X046			A2007100X046	A2014100X046	A2005100X046
4.6 x 75	3	A2001075X046	A2011075X046					

*Normal phase columns.

(Continued)

Polaris HPLC Columns

Size (mm)	Particle Size (µm)	Polaris C18-A	Polaris C8-A	Polaris C18-Ether	Polaris C8-Ether	Polaris Amide C18	Polaris NH2*	Polaris Si-A*
4.6 x 50	3	A2001050X046		A2021050X046	A2031050X046	A2007050X046	A2014050X046	A2005050X046
4.6 x 30	3	A2001030X046						
4.0 x 250	5	A2000250X040	A2010250X040	A2020250X040	A2030250X040		A2013250X040	A2003250X040
4.0 x 150	5	A2000150X040	A2010150X040	A2020150X040	A2030150X040		A2013150X040	A2003150X040
4.0 x 125	5	A2000125X040	A2010125X040	A2020125X040	A2030125X040		A2013125X040	A2003125X040
3.0 x 250	5	A2000250X030	A2010250X030	A2020250X030	A2030250X030	A2006250X030	A2013250X030	A2005250X046
3.0 x 150	5	A2000150X030	A2010150X030	A2020150X030	A2030150X030	A2006150X030	A2013150X030	A2003150X030
3.0 x 100	5	A2000100X030	A2010100X030	A2020100X030	A2030100X030	A2006100X030	A2013100X030	A2003100X030
3.0 x 50	5	A2000050X030						A2003050X030
3.0 x 250	3	A2001250X030				A2007250X030	A2014250X030	A2003250X030
3.0 x 200	3	A2001200X030						
3.0 x 150	3	A2001150X030		A2021150X030		A2007150X030	A2014150X030	A2005150X030
3.0 x 100	3	A2001100X030				A2007100X030	A2014100X030	A2005100X030
3.0 x 50	3	A2001050X030		A2021050X030	A2031050X030	A2007050X030	A2014050X030	A2005050X030
3.0 x 30	3	A2001030X030	A2011030X030					
2.0 x 250	5	A2000250X020		A2020250X020	A2030250X020	A2006250X020	A2013250X020	A2003250X020
2.0 x 150	5	A2000150X020	A2010150X020	A2020150X020	A2030150X020	A2006150X020	A2013150X020	A2003150X020
2.0 x 100	5	A2000100X020				A2006100X020	A2013100X020	A2003100X020
2.0 x 50	5	A2000050X020	A2010050X020	A2020050X020	A2030050X020	A2006050X020	A2013050X020	A2003050X020
2.0 x 30	5	A2000030X020				A2006030X020	A2013030X020	A2003030X020
2.0 x 20	5	A2000020X020					A2013020X020	A2003020X020
2.0 x 250	3	A2001250X020	A2011250X020	A2021250X020	A2031250X020	A2007250X020	A2014250X020	A2005250X020
2.0 x 150	3	A2001150X020	A2011150X020	A2021150X020	A2031150X020	A2007150X020	A2014150X020	A2005150X020
2.0 x 100	3	A2001100X020		A2021100X020	A2031100X020	A2007100X020	A2014100X020	A2005100X020
2.0 x 75	3			A2021075X020				
2.0 x 50	3	A2001050X020	A2011050X020	A2021050X020	A2031050X020	A2007050X020	A2014050X020	A2005050X020
2.0 x 30	3	A2001030X020		A2021050X020		A2007030X020	A2014030X020	A2005030X020
2.0 x 20	3	A2001020X020					A2014020X020	A2005020X020

*Normal phase columns.

Polaris ChromSep Complete Cartridge Systems

Hardware	Size (mm)	Particle Size (µm)	Polaris C18-A
CS	4.6 x 250	5	A2000250C046
CS	4.6 x 150	5	A2000150C046
CS	4.6 x 100	5	A2000100C046
CS	4.6 x 250	3	A2001250C046
CS	4.6 x 150	3	A2001150C046
CS	3.0 x 250	5	A2000250C030
CS	3.0 x 100	5	A2000100C030
CS	2.0 x 100	5	A2000100C020
CS	2.0 x 150	3	A2001150C020
CS	2.0 x 100	3	A2001100C020
CS	2.0 x 50	3	A2001050C020

Polaris ChromSep Replacement Cartridges

Hardware	Size (mm)	Particle Size (µm)	Unit	Polaris C18-A
CS	4.6 x 250	5		A2000250R046
			3/pk	A2000250T046
CS	4.6 x 150	5		A2000150R046
			3/pk	A2000150T046
CS	4.6 x 100	5		A2000100R046
			3/pk	A2000100T046
CS	4.6 x 150	3		A2001150R046
			3/pk	A2001150T046
CS	4.6 x 100	3		A2001100R046
			3/pk	A2001100T046
CS	3.0 x 150	5		A2000150R030
			3/pk	A2000150T030
CS	3.0 x 100	5		A2000100R030
			3/pk	A2000100T030
CS	3.0 x 100	3		A2001100R030
			3/pk	A2001100T030
CS	2.0 x 150	3		A2001150R020
			3/pk	A2001150T020
CS	2.0 x 50	3		A2001050R020
			3/pk	A2001050T020

MetaGuard Columns

Hardware	Dimensions	Particle Size (µm)	Polaris C18-A	Polaris C8-A	Polaris C18-Ether	Polaris C8-Ether	Polaris Amide C18	Polaris NH2*	Polaris Si-A*
MG	4.6	10	A2002MG						A2004MG
MG	2.0	10					A2008MG2		A2004MG2
MG	4.6	5	A2000MG	A2010MG	A2020MG	A2030MG	A2006MG	A2013MG	A2003MG
MG	2.0	5	A2000MG2	A2010MG2	A2020MG2		A2006MG2	A2013MG2	A2003MG2
MG	4.6	3	A2001MG	A2011MG	A2021MG		A2007MG	A2014MG	A2005MG
MG	2.0	3	A2011MG2	A2011MG2	A2021MG2	A2031MG2	A2007MG2	A2014MG2	A2005MG2
MG	1.0	3	A2001MG1						

*Normal phase columns.

Agilent TC-C18(2) and HC-C18(2)

For cost-conscious chromatographers who need traditional LC columns and don't need the individual testing of ZORBAX, Pursuit or Polaris columns, the Agilent TC(2)/HC(2) columns provide an alternative.

TC-C18(2)

Agilent TC-C18(2) is the ideal choice for complex natural product extract samples, traditional medicines and environmental samples or any sample where you need to analyze mixtures of polar and non-polar compounds, including strong basic compounds.

- Lower carbon load – 12%
- Ideal for polar compounds and gradient separations that start at low % organic or cover a wide organic range
- Good choice for samples dissolved in water, or mostly water
- Use with most common mobile phases, including formic acid, acetic acid, trifluoroacetic acid (TFA) and phosphate buffers with acetonitrile and methanol as the organic modifiers
- Excellent performance from pH 2-8

HC-C18(2)

Agilent HC-C18(2) is a more retentive C18 with a higher carbon load. An excellent value alternative to other high carbon load columns, it also provides superior peak shape for basic compounds.

- Higher carbon load – 17% – provides greater retention for moderately polar and non-polar compounds
- Ideal for non-polar compounds and separations that start at mid-level % organic (at least greater than 10% organic)
- Good choice for industrial samples or samples dissolved in organic/mostly organic solvents
- Stable over a very wide pH range (2-9) for maximum flexibility

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits	pH Range*	Endcapped	Carbon Load
TC-C18(2)	170Å	290 m^2/g	60 °C	2.0-8.0	Yes	12%
HC-C18(2)	170Å	290 m^2/g	60 °C	2.0-9.0	Yes	17%

Specifications represent typical values only.

Agilent HC-C18(2) and TC-C18(2)

Description	Size (mm)	Particle Size (µm)	Part No.
Agilent HC-C18(2)	4.6 x 250	5	588905-902
Agilent HC-C18(2)	4.6 x 150	5	588915-902
Agilent TC-C18(2)	4.6 x 250	5	588925-902
Agilent TC-C18(2)	4.6 x 150	5	588935-902
Agilent HC-C18(2) guards, 2/pk	4.6 x 12.5	5	520518-904
Agilent TC-C18(2) guards, 2/pk	4.6 x 12.5	5	520518-905
Guard Hardware Kit			820999-901

TIPS & TOOLS

Don't forget, we have special offers throughout the year.

To learn more, visit **www.agilent.com/chem/specialoffers**

PLRP-S HPLC Columns

- Contain durable and resilient polymer particles that deliver reproducible results over longer lifetimes
- Thermally and chemically stable
- Comply with USP L21 designation
- Used in bioscience, chemical, clinical research, energy, environmental, food and agriculture, material science and pharmaceutical industries
- Pore sizes (100Å-4000Å) for separations of small molecules to large complexes and polynucleotides

The PLRP-S family of columns consists of a range of pore sizes and particle sizes, all with identical chemistry and fundamental adsorptive characteristics. The particles are inherently hydrophobic, therefore no bonded phase, alkyl ligand is required for reversed-phase separations. This gives a highly reproducible material that is free from silanols and heavy metal ions. Columns within the extensive product range are suitable for micro separations, including both bottom-up and top-down proteomics, analytical separations, and preparative purifications. In addition, process columns can be packed with bulk media.

Column Specifications	
pH Range	1-14
Buffer Content	Unlimited
Organic Modifier	1-100%
Temperature Limits	200 °C
Maximum Pressure	5-8 µm: 3000 psi (210 bar)
	3 µm: 4000 psi (300 bar)

PLRP-S Applications	
Pore Size	**Application**
100Å	Small molecules/synthetic biomolecules
300Å	Recombinant peptides/proteins
1000Å	Large proteins
4000Å	DNA/high speed

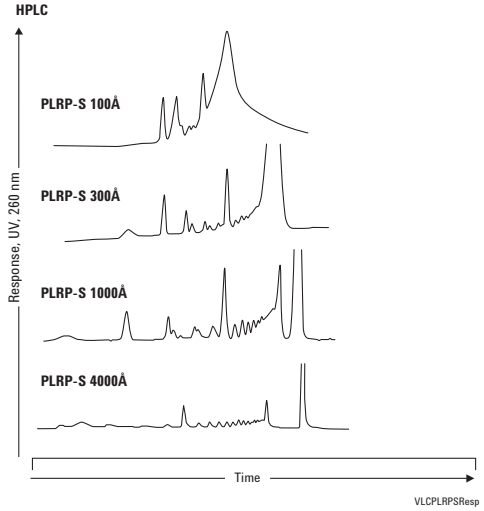
HPLC of 25 bp DNA ladder

Column: **PLRP-S, 2.1 x 150 mm**

Mobile Phase: A: 0.1 M TEAA
B: 0.1 M TEAA in 50% water:50% ACN

Flow Rate: 200 µL/min

Gradient: 12.5-50% B in 150 min



Polyethylene glycols

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: A: Water
B: ACN

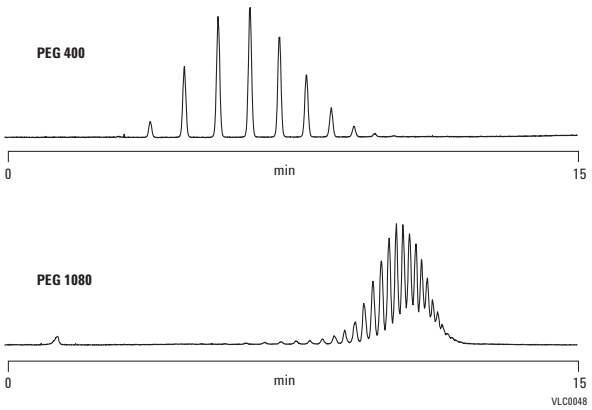
Gradient: 10-30% B in 12 min, held at 30% B for 3 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

Detector: ELS (neb=50 °C, evap=70 °C, gas=1.6 SLM)



Exploiting chemical stability – NH_4OH concentration

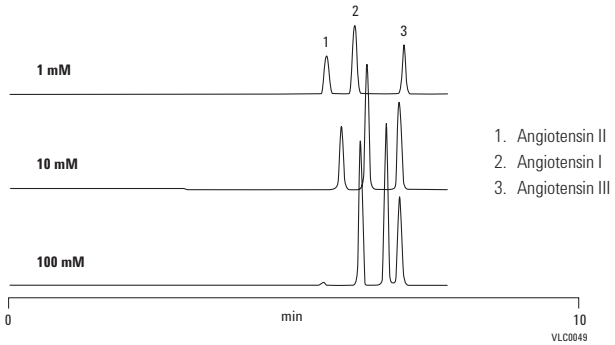
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: A: NH_4OH (various mM) in water
B: NH_4OH (various mM) in ACN

Gradient: Linear 10-100% B in 15 min

Flow Rate: 1.0 mL/min

Detector: ELS (neb=80 °C, evap=85 °C, gas=1.0 SLM)



1. Angiotensin II
2. Angiotensin I
3. Angiotensin III

Alberta Peptide Institute test mix

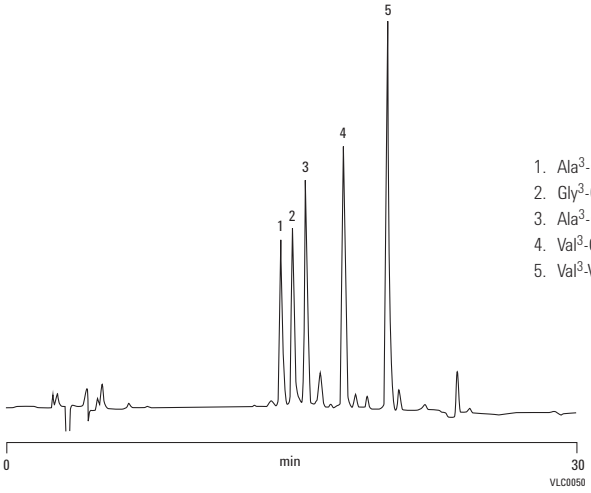
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 70% water:30% ACN

Gradient: 0-100% B in 30 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



1. Ala^3-Gly^4 (free amino)
2. Gly^3-Gly^4 (Nα-acetylated)
3. Ala^3-Gly^4 (Nα-acetylated)
4. Val^3-Gly^4 (Nα-acetylated)
5. Val^3-Val^4 (Nα-acetylated)

Large fibrous proteins

Column: **PLRP-S 300Å**
PL1512-3801
4.6 x 150 mm, 8 µm

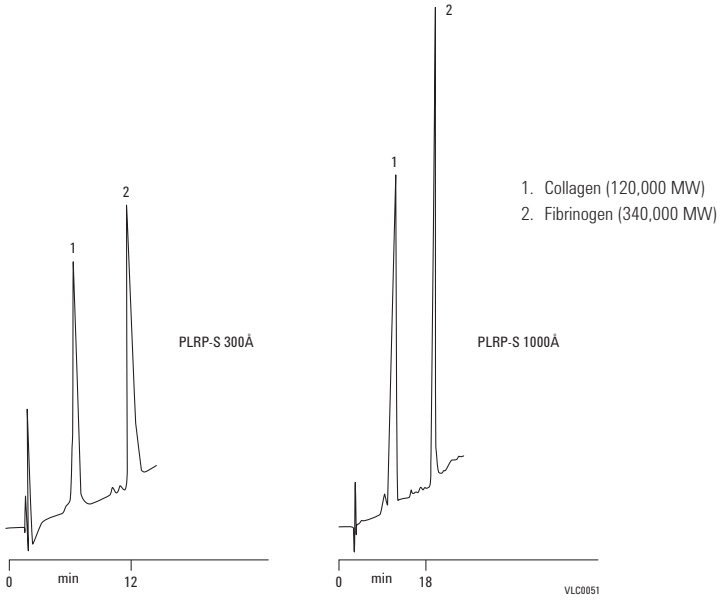
Column: **PLRP-S 1000Å**
PL1512-3802
4.6 x 150 mm, 8 µm

Mobile Phase: A: 0.25% TFA in water
B: 0.25% TFA in 5% water:95% ACN

Flow Rate: 1.0 mL/min

Gradient: 20-60% B in 15 min

Detector: UV, 220 nm



1. Collagen (120,000 MW)
2. Fibrinogen (340,000 MW)

PLRP-S HPLC Columns

Hardware	Size (mm)	Particle Size (µm)	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
	4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
	4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
	4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
	4.6 x 250	5	PL1512-5500	PL1512-5501		
	4.6 x 150	5	PL1111-3500	PL1512-3501		
	4.6 x 50	5	PL1512-1500	PL1512-1501	PL1512-1502	PL1512-1503
	4.6 x 150	3	PL1512-3300	PL1512-3301		
	4.6 x 50	3	PL1512-1300	PL1512-1301		
	2.1 x 250	8		PL1912-5801		
	2.1 x 150	8		PL1912-3801	PL1912-3802	PL1912-3803
	2.1 x 50	8		PL1912-1801	PL1912-1802	PL1912-1803
	2.1 x 250	5	PL1912-5500	PL1912-5501		
	2.1 x 150	5	PL1912-3500	PL1912-3501		
	2.1 x 50	5	PL1912-1500	PL1912-1501	PL1912-1502	PL1912-1503
	2.1 x 150	3	PL1912-3300	PL1912-3301		
	2.1 x 50	3	PL1912-1300	PL1912-1301		
PL	PLRP-S Guard Cartridges for 5 x 3 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
PL	Guard Cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

*Prep columns are also available for the PLRP-S family. Turn to pages 467-471

Preparative HPLC Columns

Flexible, cost-effective options for scaling and prep

Whether you are scaling up a routine analytical method, or maintaining precise separations throughout every phase of production, Agilent can help you rise to the challenge.

- Agilent Prep LC columns are a cost-effective prep solution designed for high loadability to purify milligram to gram quantities of product
- ZORBAX PrepHT columns are designed for rapid scale-up from the ZORBAX family of phases
- Scalable prep columns are also available for Pursuit and Polaris columns
- Bulk materials are available for all phases and can be ordered through Agilent's Custom Ordering Process, **www.agilent.com/chem/customlc**

Prep LC Columns

Agilent Prep LC Columns

- High loadability for maximum sample purification
- Easy scalability from 4.6 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 size 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

Specifications represent typical values only.

*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.

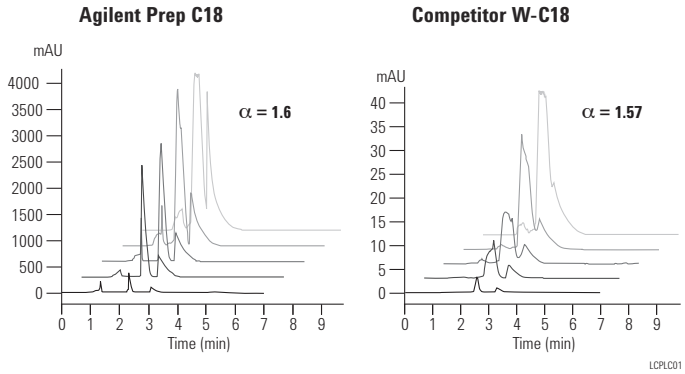
Superior loadability on Agilent Prep C18 with basic compounds

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

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 150 mm, 5 µm**

Column B: **Agilent Prep C18 443905-102 21.2 x 150 mm, 5 µm**

Column C: **Agilent Prep C18 413910-302 30.0 x 150 mm, 10 µm**

Column D: **Agilent Prep C18 413910-502 50.0 x 150 mm, 10 µm**

Mobile Phase: 55% Water:45% ACN

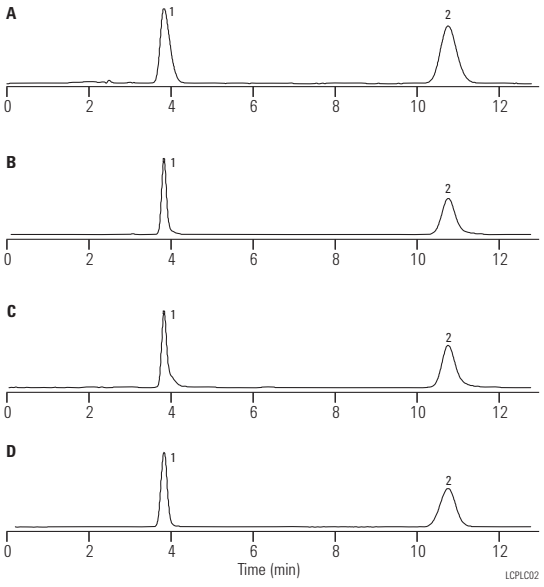
Flow Rate: 0.7 mL/min
14.87 mL/min
29.77 mL/min
85.37 mL/min

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 Size (µm)	C18	Silica
Standard Columns (no special hardware required)					
	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)					
	Prep 30	30.0 x 250	10	410910-302	410910-301
	Prep 30	30.0 x 150	10	413910-302	413910-301
	Prep 30	30.0 x 100	10	419910-302	419910-301
	Prep 30	30.0 x 100	5	449905-302	449905-301
	Prep 30	30.0 x 50	5	446905-302	446905-301
	Prep 50	50.0 x 250	10	410910-502	410910-501
	Prep 50	50.0 x 150	10	413910-502	413910-501
	Prep 50	50.0 x 100	10	419910-502	419910-501
	Prep 50	50.0 x 100	5	449905-502	449905-501
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 (1 kg)		10	420910-902	420910-901

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

ZORBAX PrepHT Columns

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
- Use to maintain selectivity of the analytical phase in your prep separations

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.

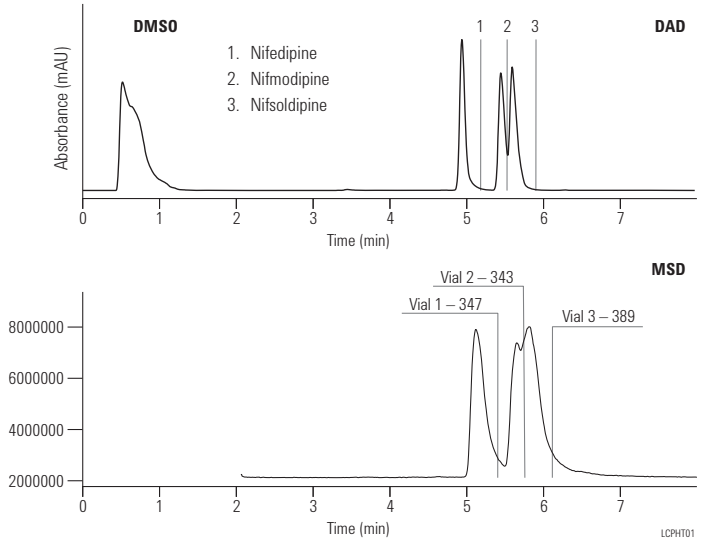
ZORBAX PrepHT columns are packed with 5 and 7 µm particle sizes for very high resolution. The high resolution allows high loadability, high yield, and high purity of compounds. The larger diameter columns and mechanically stronger ZORBAX particles allow for flow rates up to 100 mL/min, thus increasing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



	Amount Nifedipine [mg]	Amount Nifmodipine [mg]	Amount Nifsoldipine [mg]		
Fraction 1	18.90	0.11	0.16	Purity Nifedipine	98.6%
Fraction 2	0.29	17.66	0.77	Purity Nifmodipine	94.4%
Fraction 3	0.49	1.66	18.36	Purity Nifsoldipine	89.5%
Recovery [mg]	19.68	19.43	19.29		
Recovery [%]	101.3	102.0	101.9		

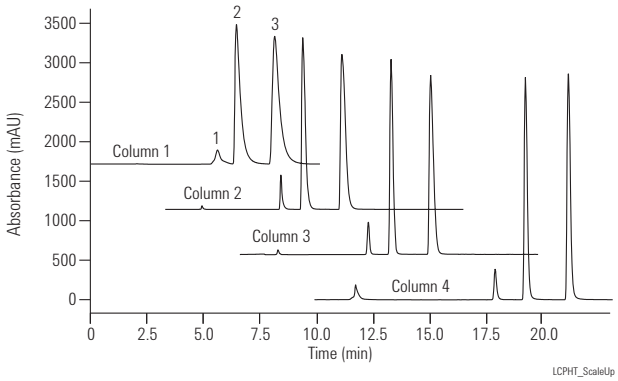
ZORBAX PrepHT columns are designed for rapid scale-up from analytical to preparative scale without losing resolution. For complex separations for larger columns (21.2 mm id and higher, 150 mm length and higher), Agilent has carefully chosen the 7 µm particle size to achieve a balance between high efficiency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

Scale-up from analytical to prep ZORBAX SB-C18 columns using the same pump

Column	Size	Flow (mL/min)	Injection (µL)	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 SS	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 increases throughput by reducing the time required for redeveloping and adjusting the method.

Scale-up to PrepHT



1. Theobromine
2. Theophylline
3. Caffeine

ZORBAX PrepHT 80StableBond (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	SB-C18 USP L1	SB-C8 USP L7	SB-Aq	SB-CN USP L10	SB-Phenyl USP L11
	PrepHT Cartridge	21.2 x 250	7	877250-102	877250-106	877250-114	877250-105	877250-112
	PrepHT Cartridge	21.2 x 150	7	877150-102	877150-106	877150-114		
	PrepHT Cartridge	21.2 x 150	5	870150-902	870150-906	870150-914		
	PrepHT Cartridge	21.2 x 100	5	870100-902	870100-906	870100-914		
	PrepHT Cartridge	21.2 x 50	5	870050-902	870050-906	870050-914		
	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-920	820212-915	820212-933	820212-933	820212-915

ZORBAX PrepHT 300StableBond (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-C3 USP L56	300SB-CN USP L10
	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-109	897250-105
	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106	897150-109	
	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906	895150-909	
	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906	895100-909	
	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906	895050-909	
	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924
	Guard Cartridge Hardware Includes guard column end fitting, polymeric seal, and seal insertion tool (seal holder and seal pusher)			820444-901	820444-901	820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901

ZORBAX PrepHT Original (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	ODS (C18) USP L1	C8 USP L7	CN USP L10	NH2 USP L8	SIL USP L3
	PrepHT Cartridge	21.2 x 250	7	877952-102	877952-106	877952-105	877952-108	877952-101
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901	820400-901

ZORBAX PrepHT Eclipse XDB (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	Eclipse XDB-C18 USP L1	Eclipse XDB-C8 USP L7
PL	PrepHT Cartridge	21.2 x 250	7	977250-102	977250-106
PL	PrepHT Cartridge	21.2 x 150	7	977150-102	977150-106
PL	PrepHT Cartridge	21.2 x 150	5	970150-902	970150-906
PL	PrepHT Cartridge	21.2 x 100	5	970100-902	970100-906
PL	PrepHT Cartridge	21.2 x 50	5	970050-902	970050-906
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-925	820212-926
	Guard Cartridge Hardware Includes guard column end fitting, polymeric seal, and seal insertion tool (seal holder and seal pusher)			820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901

ZORBAX PrepHT Bonus-RP and Extend-C18 (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	Bonus-RP USP L60	Extend-C18 USP L1
PL	PrepHT Cartridge	21.2 x 250	7	878250-101	
PL	PrepHT Cartridge	21.2 x 150	7	878150-101	
PL	PrepHT Cartridge	21.2 x 150	5	868150-901	770150-902
PL	PrepHT Cartridge	21.2 x 100	5	868100-901	770100-902
PL	PrepHT Cartridge	21.2 x 50	5	868050-901	770050-902
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-928	820212-930
	Guard Cartridge Hardware Includes guard column end fitting, polymeric seal, and seal insertion tool (seal holder and seal pusher)			820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901

ZORBAX PrepHT Rx-SIL (require hardware 820400-901)

Hardware	Description	Size (mm)	Particle Size (µm)	Rx-SIL USP L3	Rx-C18 USP L1
PL	PrepHT Cartridge	21.2 x 250	7	877250-101	
PL	PrepHT Cartridge	21.2 x 250	7		877967-102
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-919	820212-914
	Guard Cartridge Hardware Includes guard column end fitting, polymeric seal, and seal insertion tool (seal holder and seal pusher)			820444-901	820444-901
	PrepHT Endfittings, 2/pk			820400-901	820400-901

ZORBAX PrepHT Accessories

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

Pursuit and Pursuit XRs Prep

- Prep-scalable columns for Pursuit and Pursuit XRs columns
- Particle sizes to 10 µm and column diameters up to 50 mm
- High surface area silica

Pursuit and Pursuit XRs Prep columns are designed for high loadability with a high surface area.

Natural products – capsaicin and dihydrocapsaicin on Pursuit XRs C18

Column A: **Pursuit XRs C18**
A6001150X046
4.6 x 150 mm, 3 µm

Column B: **Pursuit XRs C18**
A6000150X046
4.6 x 150 mm, 5 µm

Column C: **Pursuit XRs C18**
A3002150X046
4.6 x 150 mm, 10 µm

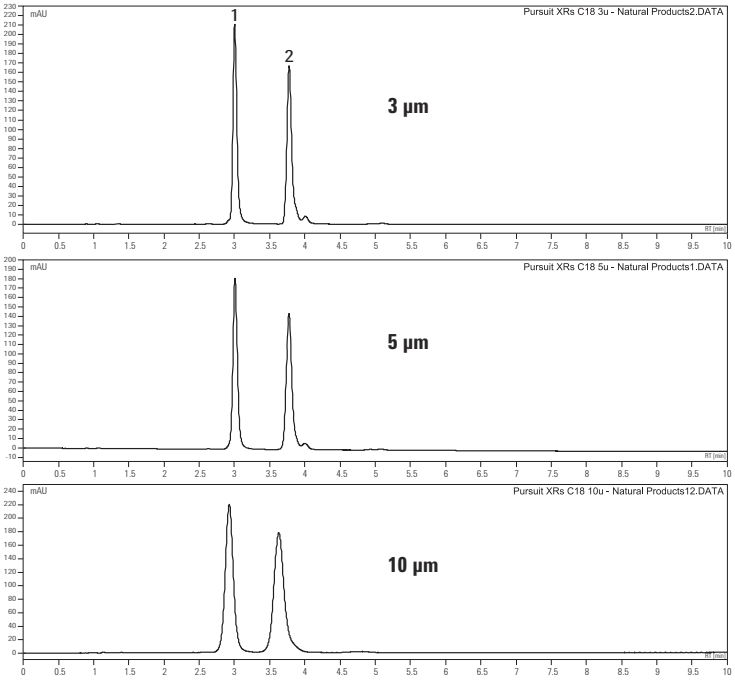
Mobile Phase: $CH_3CH:H_20$ - 70:30

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 220 nm

Sample: 1. Capsaicin
2. Dihydrocapsaicin



Demonstrating an easy, linear scale-up of natural products from Pursuit XRs C18 3 µm and 5 µm analytical columns to a 10 µm preparative column.

Agilent Pursuit Prep Columns

Size (mm)	Particle Size (µm)	Pursuit C18 USP L1	Pursuit C8 USP L7	Pursuit Diphenyl	Pursuit PFP
10.0 x 250	5	A3000250X100	A3030250X100	A3040250X100	A3050250X100
10.0 x 250	10	A6002250X100	A3032250X100		
21.2 x 250	10	A6002250X212			
21.2 x 250	10	A6002250X212	A3032250X212		

Agilent Pursuit XRs Prep Columns

Size (mm)	Particle Size (µm)	Pursuit XRs C18 USP L1	Pursuit XRs C8 USP L7	Pursuit XRs Diphenyl	Pursuit XRs Si USP L3
21.2 x 250	10	A6002250X212			A6004250X212
21.2 x 250	5	A6000250X212		A6020250X212	
21.2 x 150	5	A6000150X212	A6010150X212		
21.2 x 100	5	A6000100X212	A6010100X212	A6020100X212	
21.2 x 50	5	A6000050X212			
30.0 x 250	10	A6002250X300			A6004250X300
30.0 x 150	10	A6002150X300			
30.0 x 250	5	A6000250X300	A6010250X300		
30.0 x 150	5	A6000150X300			
30.0 x 100	5	A6000100X300			
50.0 x 250	10	A6002250X500		A6022250X500	A6004250X500

Polaris Prep Columns

- Prep-scalable columns for Polaris phases
- 10.0 and 21.2 mm ids available, with particles up to 10 µm

Scale-up from analytical to prep

Column A: **Polaris C18-A**
A2001050X046
4.6 x 50 mm, 3 µm

Column B: **Polaris C18-A**
A2000050X046
4.6 x 50 mm, 5 µm

Column C: **Custom column**

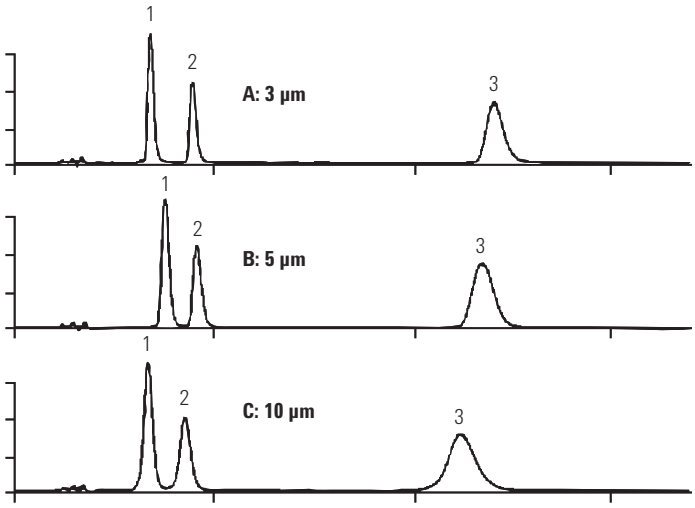
Mobile Phase: A: H_20 + 0.1% NH_40H, pH 10.0 (25 mM)
B: CH_3CN
A:B 65:35

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: 215 nm

Sample: 1. Pindolol
2. Metoprolol
3. Propranolol



Polaris Prep Columns

Size (mm)	Particle Size (µm)	Polaris C18-A	Polaris C18-Ether	Polaris Amide C18	Polaris Si-A	Polaris C8-A	Polaris C8-Ether	Polaris NH2
10.0 x 250	5	A2000250X100	A2020250X100	A2006250X100		A2010250X100	A2030250X100	A2013250X100
21.2 x 250	5	A2000250X212	A2030250X212		A2003250X212	A2010250X212		A2013250X212
21.2 x 250	10	A2002250X212			A2004250X212			

Bulk materials for prep

Agilent has bulk materials available for all phases. Most materials and quantities can be ordered through the Custom Column and Bulk Ordering process, and can be produced in multiple kg. quantities. Quotes are able to be delivered within 48 hours. Contact your Agilent product specialist for support in placing a custom order.

Load & Lock Columns

Load & Lock Preparative HPLC Column Packing Systems

Agilent offers a complete range of Load & Lock column systems for laboratory and process preparative LC. They are designed to let you easily and quickly pack your own preparative high efficiency columns. This is the right solution for applications ranging in scale from development (multigrams) to production (multi-kilo) of pharmaceutical compounds, peptides, and natural products. Our Load & Lock columns have a unique fluid/sample distribution system to maximize productivity. The system provides dynamic axial compression (DAC) and static "locked" axial compression (SAC) and is designed for easy operation to deliver greater convenience.

Laboratory Load & Lock Columns

- Mobile packing station supports three different column sizes
- Runs on compressed air with no need for a power supply
- Quick and easy packing and unpacking within minutes

Agilent's laboratory scale Load & Lock columns combine excellent packed-bed stability with enhanced flow distribution to deliver the highest quality purification possible with maximum speed, flexibility, and ease of operation. Three different column sizes are supported: 1 in, 2 in and 3 in id. Because the station is powered by compressed air, it is the perfect solution for hazardous environments. The quick-release single bolt clamp offers speedy and easy packing and unpacking within minutes.

Load & Lock Preparative HPLC Column Packing Systems

Description	Water Jacket	Size (mm)	Part No.
Load & Lock 4001 Column	No	25.0 x 500	PCG93LL500X25
	Yes	25.0 x 500	PCG93LL500X25WJ
	Spare parts kit		PCG931AAKIT
Load & Lock 4002 Column	No	50.0 x 500	PCG93LL500X50
	Yes	50.0 x 500	PCG93LL500X50WJ
	Spare parts kit		PCG932AAKIT
Load & Lock 4003 Column	No	75.0 x 500	PCG93LL500X75
	Yes	75.0 x 500	PCG93LL500X75WJ
	Spare parts kit		PCG933AAKIT
Mobile packing station (air driven hydraulic)			PCG93LLSTAND123

Columns for Other HPLC Techniques

Reproducible results for Normal Phase and beyond

Agilent's extended family of HPLC columns support every technique, providing you with the Agilent quality you depend on for every application.

- ZORBAX HILIC Plus – good retention of small, polar analytes and high sensitivity for LC/MS – in Fast LC 1.8 µm options
- ZORBAX normal phase columns – bonded and non-bonded silica packings
- ZORBAX ion-exchange columns – based on rugged ZORBAX Silica, stable from pH 2-7
- Hi-Plex columns for carbohydrate analysis – ligand-exchange columns
- Ultron ES Chiral columns – with two complimentary protein-based chiral stationary phases – are an excellent choice for enantiomeric separations. Ideal for many pharmacological applications.

ZORBAX HILIC Plus

- HILIC column for good retention of small, polar analytes
- Based on Eclipse Plus silica for excellent peak shape
- High sensitivity for LC/MS applications
- Recommended for EPA Method 1694

Agilent ZORBAX HILIC Plus columns are for use in hydrophilic interaction chromatography (HILIC) applications, which are typically used for the retention and resolution of small polar compounds. HILIC Plus columns are non-bonded silica columns based on the high performance silica used in ZORBAX Eclipse Plus columns. This silica provides excellent peak shape, critical for many polar, basic analytes. These columns ship prepared for use in HILIC mode – containing acetonitrile:water – in order to reduce the extensive equilibration typically required for HILIC separations. HILIC Plus columns are available in a 3.5 µm particle size for high resolution and in 2.1 and 4.6 mm id for compatibility with mass spectrometers or with standard UV detectors.

Column Specifications

Phase	Pore Size	Surface Area	pH Range
Non-bonded silica	95Å	160 m^2/g	0-8.0

Specifications represent typical values only.

TIPS & TOOLS

Poroshell 120 HILIC is very similar to ZORBAX HILIC Plus.

Turn to page 228

Separation of group 4 analytes in EPA 1694 on ZORBAX HILIC Plus column

Column: **ZORBAX HILIC Plus**
959793-901
2.1 x 100 mm, 3.5 µm

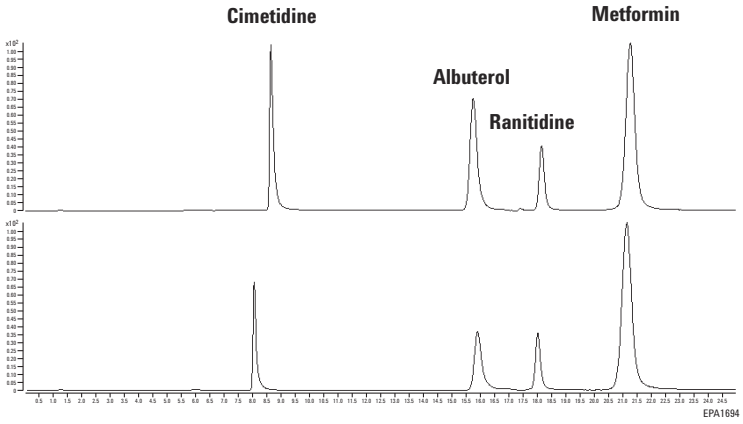
Mobile Phase: 90% Acetontrile:10% Water

Flow Rate: 0.25 mL/min

Gradient: Linear gradient to 55% acetonitrile in 7 min
Held at 55%

Temperature: 25 °C

Duplicate runs for column USCJP0004;
10 min equilibration between two runs



ZORBAX HILIC Plus

Description	Size (mm)	Particle Size (µm)	Part No.
Analytical	4.6 x 100	3.5	959961-901
Analytical	4.6 x 50	3.5	959943-901
Narrow Bore	2.1 x 100	3.5	959793-901
Narrow Bore	2.1 x 50	3.5	959743-901

ZORBAX HILIC Plus RRHD, stable to 1200 bar

Size (mm)	Particle Size (µm)	Part No.
3.0 x 100	1.8	959758-301
3.0 x 50	1.8	959757-301
2.1 x 150	1.8	959759-901
2.1 x 100	1.8	959758-901
2.1 x 50	1.8	959757-901

Poroshell 120 HILIC Plus

Size (mm)	Particle Size (µm)	Part No.
2.1 x 50	2.7	699775-901
2.1 x 100	2.7	695775-901
2.1 x 150	2.7	693775-901
3 x 50	2.7	699975-301
3 x 150	2.7	693975-301
4.6 x 50	2.7	699975-901
4.6 x 100	2.7	695975-901
4.6 x 150	2.7	693975-901

ZORBAX Normal-Phase Columns

For normal-phase chromatography, the Agilent 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)
- Available in 1.8 and 5 µm particle sizes
- 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

- Cyanopropyldimethylsilane 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

Pursuit XRs Si

- 100Å silica for higher surface area and good loadability
- 14.6% carbon load
- Available in 3 µm, 5 µm and 10 µm

Polaris NH2

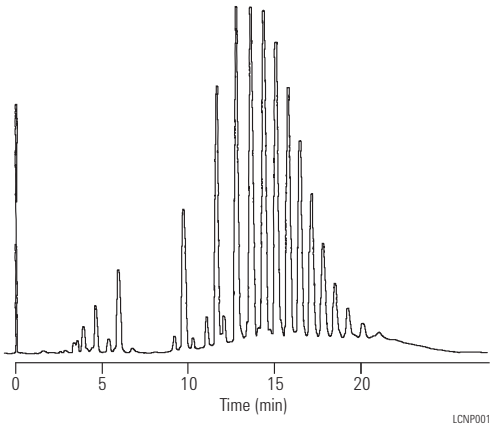
- 180Å silica for high surface area and loadability
- 5.5% carbon load
- Available in 3 µm, 5 µm, and 10 µm sizes
- Polar-modified with silanol shielding
- Designed for high-aqueous conditions

Polaris Si-A

- 180Å silica with highest surface area and loadability
- Available in 3 µm, 5 µm, and 10 µm

High resolution normal-phase separation of octylphenoxy ethanol surfactant on ZORBAX CN

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



Polaris ordering information can be found on pages 300-303

Pursuit ordering information can be found on pages 291-297

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

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 NH2	70Å	300 m^2/g	2.0-7.0	Yes	4%

TIPS & TOOLS

Pursuit XRs Silica is another choice for normal-phase chromatography. For more information, see pages 295-296.

Normal-Phase Columns Based on ZORBAX Rx-SIL

Hardware	Description	Size (mm)	Particle Size (µm)	Rx-SIL** USP L3	Eclipse XDB-CN USP L10
Standard Columns (no special hardware required)					
	Semi-Prep	9.4 x 250	5	880975-201	
	Analytical	4.6 x 250	5	880975-901	990967-905*
	Analytical	4.6 x 150	5	883975-901	993967-905*
	Rapid Resolution HT, 600 bar	4.6 x 100	1.8	828975-901	
	Rapid Resolution HT, 600 bar	4.6 x 50	1.8	827975-902	
	Rapid Resolution HT, 600 bar	3.0 x 100	1.8	828975-301	
	Rapid Resolution HT, 600 bar	3.0 x 50	1.8	827975-301	
	Narrow Bore	2.1 x 150	5	883700-901	993700-905*
	Rapid Resolution HT, 600 bar	2.1 x 100	1.8	828700-901	
	Rapid Resolution HT, 600 bar	2.1 x 50	1.8	827700-901	
Guard Columns (hardware required)					
P	Guard Cartridge, 2/pk	9.4 x 15	5	820675-119	
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-919	820950-935
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-919	821125-935
P	Guard Hardware Kit	9.4 x 15		840140-901	
ZGC	Guard Hardware Kit			820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)					
PH	PrepHT Cartridge	21.2 x 250	7	877250-101	
PH	PrepHT Endfittings, 2/pk			820400-901	
PH	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-919	
PH	Guard Cartridge Hardware			820444-901	

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

**These columns can also be used in HILIC mode.

Normal-Phase Columns Based on ZORBAX Original SIL

Hardware	Description	Size (mm)	Particle Size (µm)	SIL USP L3	CN USP L10	NH2 USP L8	Carbohydrate Analysis*
Standard Columns (no special hardware required)							
	Semi-Prep	9.4 x 250	5	880952-201	880952-205	880952-208	
	Analytical	4.6 x 250	5	880952-701	880952-705	880952-708	840300-908
	Analytical	4.6 x 150	5	883952-701	883952-705	883952-708	843300-908
	Narrow Bore	2.1 x 50	5			860700-708	
Guard Columns (hardware required)							
P	Guard Cartridge, 2/pk	9.4 x 15	5	820675-119	820675-111	820675-111	
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-901	820950-905	820950-908	820950-908
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5				
P	Guard Hardware Kit	9.4 x 15		840140-901	840140-901	840140-901	
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901
PrepHT Cartridge Columns (require endfittings kit 820400-901)							
PI	PrepHT Cartridge	21.2 x 250	7	877952-101	877952-105	877952-108	
PI	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	
PI	Guard Cartridge Hardware			820444-901			

*Columns ship in acetonitrile:water and are tested with a mix of sugars.

Pursuit XRs Si, USP L3

Size (mm)	Particle Size (µm)	Part No.
Semi-Prep Scale		
10.0 x 250	10	A6004250X100
Analytical Scale		
4.6 x 250	10	A6004250X046
4.6 x 100	5	A6006100X046
4.6 x 50	5	A6006050X046
4.6 x 100	3	A6005100X046
4.6 x 50	3	A6005050X046
2.1 x 100	5	A6006100X021
2.0 x 50	3	A6005050X020
Prep Scale		
50.0 x 250	10	A6004250X500
30.0 x 250	10	A6004250X300
21.2 x 250	10	A6004250X212

Polaris HPLC Columns

Size (mm)	Particle Size (µm)	Polaris NH2	Polaris Si-A
50.0 x 250	10		A2004250X500
21.2 x 250	10		A2004250X212
21.2 x 250	5	A2013250X212	A2003250X212
21.2 x 150	5		A2003150X046
21.2 x 50	5		A2003050X212
10.0 x 250	5	A2013250X100	

(Continued)

Polaris HPLC Columns

Size (mm)	Particle Size (µm)	Polaris NH2	Polaris Si-A
4.6 x 250	10		A2003250X046
4.6 x 250	5	A2013250X046	
4.6 x 150	5	A2013150X046	A2003150X046
4.6 x 100	5	A2013100X046	A2003100X046
4.6 x 50	5	A2013050X046	A2003050X046
4.6 x 250	3	A2014250X046	A2005250X046
4.6 x 150	3	A2014150X046	A2005150X046
4.6 x 100	3	A2014100X046	A2005100X046
4.6 x 50	3	A2014050X046	A2005050X046
4.0 x 250	5	A2013250X040	A2003250X040
4.0 x 150	5	A2013150X040	A2003150X040
4.0 x 125	5	A2013125X040	A2003125X040
3.0 x 250	5	A2013250X030	A2005250X046
3.0 x 150	5	A2013150X030	A2003150X030
3.0 x 100	5	A2013100X030	A2003100X030
3.0 x 50	5		A2003050X030
3.0 x 250	3	A2014250X030	A2003250X030
3.0 x 150	3	A2014150X030	A2005150X030
3.0 x 100	3	A2014100X030	A2005100X030
3.0 x 50	3	A2014050X030	A2005050X030
2.0 x 250	5	A2013250X020	A2003250X020
2.0 x 150	5	A2013150X020	A2003150X020
2.0 x 100	5	A2013100X020	A2003100X020
2.0 x 50	5	A2013050X020	A2003050X020
2.0 x 30	5	A2013030X020	A2003030X020
2.0 x 20	5	A2013020X020	A2003020X020
2.0 x 250	3	A2014250X020	A2005250X020
2.0 x 150	3	A2014150X020	A2005150X020
2.0 x 100	3	A2014100X020	A2005100X020
2.0 x 50	3	A2014050X020	A2005050X020
2.0 x 30	3	A2014030X020	A2005030X020
2.0 x 20	3	A2014020X020	A2005020X020

MetaGuard Columns

Hardware	Dimensions	Particle Size (µm)	Polaris NH2	Polaris Si-A
MG	4.6	10		A2004MG
MG	2.0	10		A2004MG2
MG	4.6	5	A2013MG	A2003MG
MG	2.0	5	A2013MG2	A2003MG2
MG	4.6	3	A2014MG	A2005MG
MG	2.0	3	A2014MG2	A2005MG2

Ion-Exchange Columns

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

Agilent 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 SAX packing has a permanently bonded quaternary amine. A trifunctional organo-silane reagent is used in producing this packing to maximize its stability with aqueous mobile phases. This column is ideal for separation of water-soluble compounds such as aromatic and aliphatic carboxylic acids and sulfonic acids.

ZORBAX SCX packing has 300Å pore size silica particles chemically bonded to an aromatic sulfonic acid group. This column is used for separations of basic, water-soluble compounds and bio-molecules.

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

Specifications represent typical values only.

Cough/cold remedies on ZORBAX 300SCX

Column: **ZORBAX 300SCX**
880952-704
4.6 x 250 mm, 5 µm

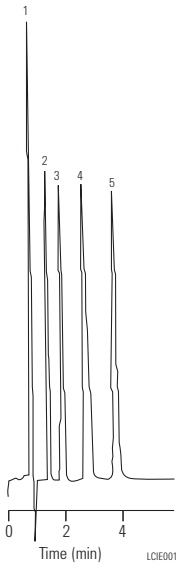
Mobile Phase: 100 mM NaH_2PO_4 (pH 6.5)

Flow Rate: 3 mL/min

Temperature: 20 °C

Detector: 210 nm

Sample: Cold remedies



1. Pyrilamine
2. Theophylline
3. Glyceryl Guaicolate
4. Caffeine
5. Phenylephrine

ZORBAX Ion-Exchange Columns – SAX and SCX

Description	Size (mm)	Particle Size (µm)	SAX	300SCX
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 250	5		880952-714*
Analytical	4.6 x 150	5	883952-703	883952-704
Analytical	4.6 x 150	5		883952-714*
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 150	5		883700-714*
Narrow Bore	2.1 x 50	5		860700-704
Guard Hardware Kit			820999-901	820999-901

*These columns have been modified to provide less retention, for those who desire that in their application.

Hi-Plex Columns for Carbohydrate Analysis

- Agilent's recommended column for accurate, low-pressure analysis of typical carbohydrates, providing leading-edge features for reliable quantitative and qualitative analysis
- Enable reduced column operating pressures for repeatable performance and longer column life
- Wide range of ligand counter ions and column configurations meet requirements of challenging organic applications
- Simplified HPLC system requirements through isocratic separation capabilities; excellent batch-to-batch reproducibility for ultimate confidence in your results
- Can be used with water or diluted acid as an eluent
- Available in 8 µm and 10 µm particle sizes in a range of choices for USP media types – L17, L19, L34 and L58

The least complicated LC methods for detecting sugars, sugar alcohols and organic acids call for ligand-exhange columns with a simple mobile phase. However, the wide particle size distribution of conventional resins can lead to high backpressures and reduced productivity.

Hi-Plex columns are engineered with monodisperse sulfonated particles, creating a high-performance media uniquely suited to stringent USP methods for analyzing carbohydrates, alcohols and organic acids. Unlike the ZORBAX NH2 column used for carbohydrate analysis with an acetonitrile:water mobile phase, Hi-Plex ligand-exchange columns provide more resolution for mono- and disaccharides due to the interaction of the hydroxyl groups with the metal ion associated with the cation-exchange functionality of the sulfonic acid group.

Column Specifications

Bonded Phase	Temperature Range	Flow Rate (mL/min)	Eluent
Hi-Plex Ca	80-90 °C	0.6	Water
Hi-Plex Ca USP L19	80-90 °C	0.3	Water
Hi-Plex Pb	70-90 °C	0.6	Water
Hi-Plex H for carbohydrates	60-70 °C	0.6	Water
Hi-Plex H for organic acids	40-60 °C	0.6	Dilute Acid
Hi-Plex Ca (Duo)	80-90 °C	0.6	Water
Hi-Plex K	80-90 °C	0.6	Water
Hi-Plex Na (Octo)	80-90 °C	0.6	Water, Sodium Hydroxide
Hi-Plex Na	80-90 °C	0.3	Water

Hi-Plex Column Selection

USP methods specify the type of HPLC media and column dimensions which should be used for the analysis. The Hi-Plex product range has four materials that comply with USP definitions.

Media Type L17

Strong cation-exchange resin consisting of sulfonated, cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter – Hi-Plex H.

Media Type L19

Strong cation-exchange resin consisting of sulfonated, cross-linked styrene-divinylbenzene copolymer in the calcium form, 9 µm in diameter – Hi-Plex Ca and Hi-Plex Ca (Duo).

Media Type L34

Strong cation-exchange resin consisting of sulfonated, cross-linked styrene-divinylbenzene copolymer in the lead form, about 9 µm in diameter – Hi-Plex Pb.

Media Type L58

Strong cation-exchange resin consisting of sulfonated, cross-linked styrene-divinylbenzene copolymer in the sodium form, 6 to 30 µm diameter – Hi-Plex Na and Hi-Plex Na (Octo).

In addition to the standard column sizes, the media is also packed in specific column dimensions for different USP methods, including sugar alcohol analysis.

For some application areas there are several column options, and the choice of the most appropriate Hi-Plex media will depend on sample matrix and exact carbohydrate composition.

Hi-Plex Column Selection	
Application Area	**Recommended Column**
USP Methods Specifying L17 Media	Hi-Plex H
USP Methods Specifying L19 Media	Hi-Plex Ca and Hi-Plex Ca (Duo)
USP Methods Specifying L34 Media	Hi-Plex Pb
USP Methods Specifying L58 Media	Hi-Plex Na and Hi-Plex Na (Octo)
Mono- and Disaccharides	Hi-Plex Ca
	Hi-Plex Pb
	Hi-Plex H
	Hi-Plex Na (Octo)
Anomer Separations	Hi-Plex Ca
Organic Acids	Hi-Plex H
Alcohols	Hi-Plex Ca
	Hi-Plex K
	Hi-Plex H
	Hi-Plex Pb
Adulteration of Food and Beverages	Hi-Plex Ca and Hi-Plex Pb
Food Additives	Hi-Plex Ca and Hi-Plex Pb
Dairy Products	Hi-Plex Ca and Hi-Plex H
Sweetened Dairy Products	Hi-Plex Pb
Confectionery	Hi-Plex Ca and Hi-Plex Pb
Fruit Juice	Hi-Plex Ca
Wine	Hi-Plex H
Wood Pulp Hydrolysates (cellulose/hemi-cellulose)	Hi-Plex Pb
Fermentation Monitoring	Hi-Plex H
Oligosaccharides	Hi-Plex Na
Samples with High Salt Content (molasses)	Hi-Plex Na (Octo)
Oligosaccharides <Dp5 with Monosaccharides	Hi-Plex Ca (Duo)
Corn Syrups	Hi-Plex Na

Analysis of fruit juice

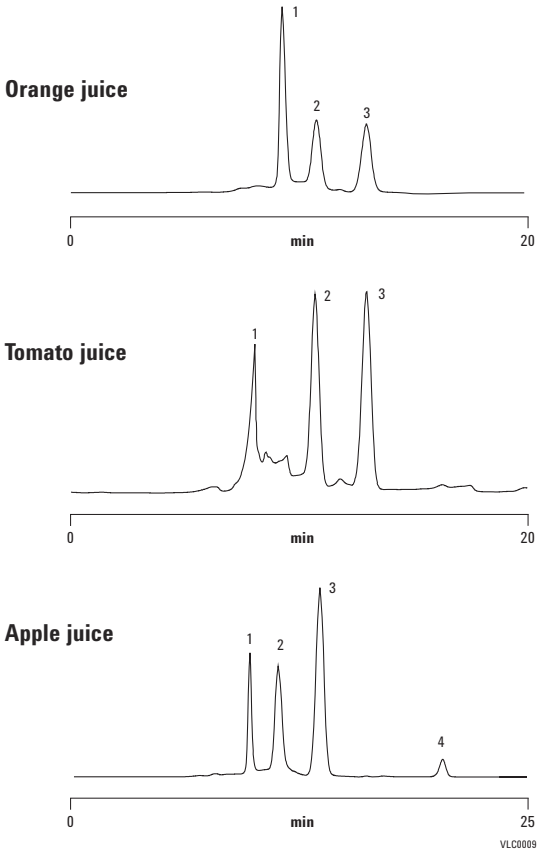
Column: **Hi-Plex Ca**
PL1170-6810
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: RI



1. Sucrose
2. Glucose
3. Fructose
4. Sorbitol

Organic acid analysis

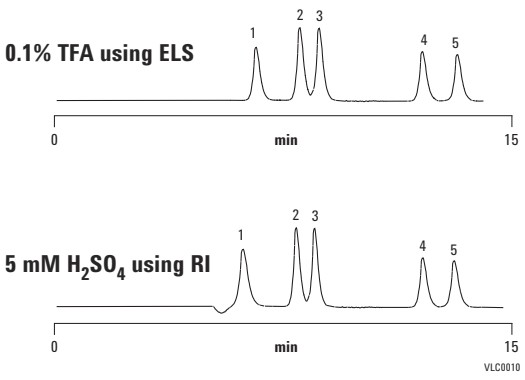
Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: Water with acid as specified

Flow Rate: 0.6 mL/min

Temperature: 60 °C

Detector: ELS (neb=80 °C, evap=90 °C, gas=0.7 SLM), RI

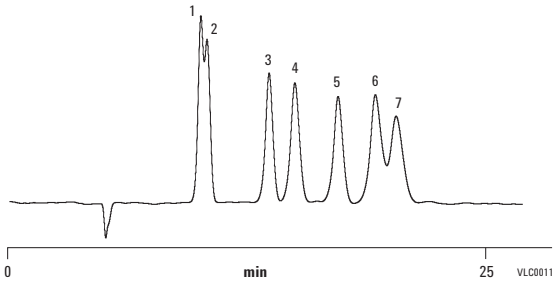


1. Oxalic acid
2. Citric acid
3. Tartaric acid
4. Succinic acid
5. Lactic acid

USP methods for sugar alcohols

Column: **Hi-Plex Ca USP L19**
PL1570-5810
4.0 x 250 mm, 8 µm

Mobile Phase: Water
Flow Rate: 0.3 mL/min
Temperature: 60 °C
Detector: RI

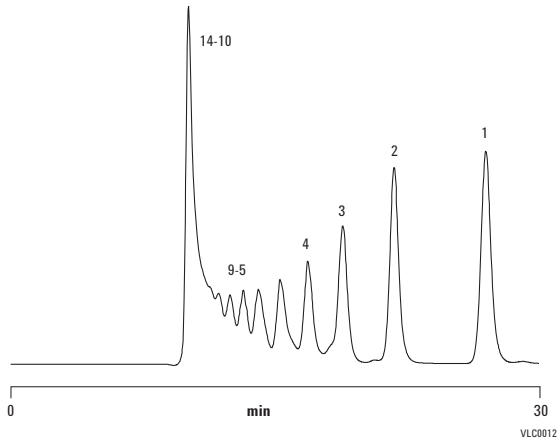


1. Iso-erythritol
2. Adonitol
3. Arabitol
4. Mannitol
5. Xylitol
6. Dulcitol
7. Sorbitol

Corn syrup, Hi-Plex

Column: **Hi-Plex Na**
PL1171-6140
7.7 x 300 mm, 10 µm

Mobile Phase: Water
Pressure: 11 bar
Flow Rate: 0.3 mL/min
Temperature: 80 °C
Detector: RI



1. Dp1
2. Dp2
3. Dp3
4. Dp4
5. Dp5
6. Dp6
7. Dp7
8. Dp8
9. Dp9
10. Dp10
11. Dp11
12. Dp12
13. Dp13
14. Dp14

Analysis of sweeteners on Hi-Plex Ca columns

Column: **Hi-Plex Ca**
PL1170-6810
7.7 x 300 mm, 8 µm

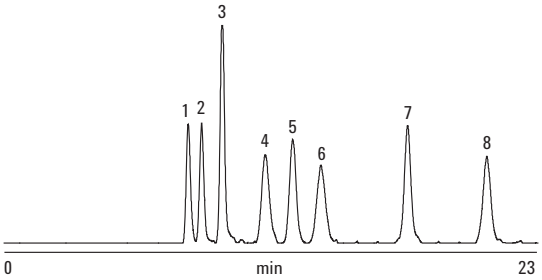
Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: ELSD

Hi-Plex Ca columns are ideal for analyzing most sweeteners, including glucose and fructose (monosaccharides), sucrose (disaccharide), and mannitol and sorbitol (sugar alcohols).



1. Stachyose
2. Raffinose
3. Sucrose
4. Glucose
5. Galactose
6. Fructose
7. Mannitol
8. Sorbitol

Analysis of carbohydates on Hi-Plex H columns

Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

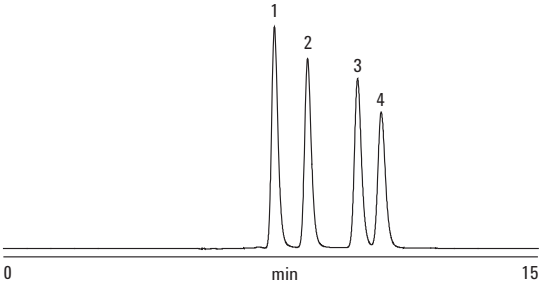
Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 70 °C

Detector: RI

For carbohydrate analysis of samples containing high levels of organic acids, Hi-Plex H columns deliver sharp, reproducible peaks. Note, however, that some sugars (such as raffinose) can undergo acid hydrolysis even when water is used as the eluent.



1. Maltotriose
2. Lactose
3. Glucose
4. Fructose

Analysis of sugars with high sodium matrix

Column: **Hi-Plex Na (Octo)**
PL1170-6840
7.7 x 300 mm, 8 µm

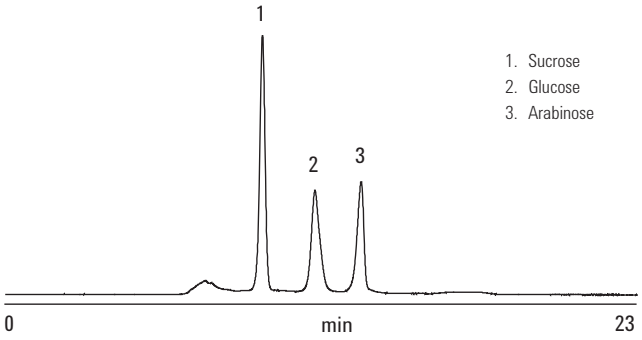
Mobile Phase: 0.015 M NaOH

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: RI

Food products containing high levels of sodium ions are best analyzed with Hi-Plex Na (Octo) columns. This saves time when sodium hydroxide is used as the eluent with PAD, because it eliminates the need for the post-column addition of sodium hydroxide.



USP method for sorbitol

Column: **Hi-Plex Pb USP L34**
PL1170-2820
7.7 x 100 mm, 8 µm

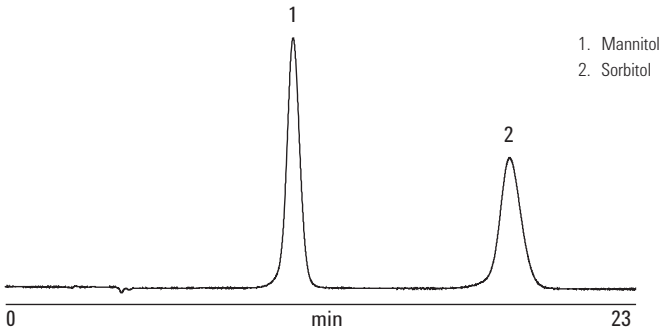
Mobile Phase: Water

Flow Rate: 0.7 mL/min

Temperature: 50 °C

Detector: RI

USP method for sorbitol – a sugar alcohol and alternative sweetener – using mannitol as the internal standard. Hi-Plex Pb columns are recommended for alcoholic drinks that also contain glycerol, as well as sweetened dairy-based food products.



Hi-Plex Columns for Carbohydrate Analysis

Description	Size (mm)	Particle Size (µm)	Crosslink Content (%)	Counter Ion	Part No.
Hi-Plex Ca USP L19	4.0 x 250	8	8	Ca^{2+}	PL1570-5810
Hi-Plex Ca (Duo)	6.5 x 300	8	8	Ca^{2+}	PL1F70-6850
Hi-Plex Ca	7.7 x 300	8	8	Ca^{2+}	PL1170-6810
Hi-Plex Pb USP L34	7.7 x 100	8	8	Pb^{2+}	PL1170-2820
Hi-Plex Pb	7.7 x 300	8	8	Pb^{2+}	PL1170-6820
Hi-Plex K	7.7 x 300	8	8	K^{+}	PL1170-6860
Hi-Plex H	6.5 x 300	8	8	H^{+}	PL1F70-6830
Hi-Plex H	7.7 x 300	8	8	H^{+}	PL1170-6830
Hi-Plex H USP L17	7.7 x 100	8	8	H^{+}	PL1170-2823
Hi-Plex Na	7.7 x 300	10	4	Na^{+}	PL1171-6140
Hi-Plex Na (Octo)	7.7 x 300	8	8	Na^{+}	PL1170-6840

Hi-Plex Guard Columns

Description	Size (mm)	Particle Size (µm)	Crosslink Content (%)	Counter Ion	Part No.
Hi-Plex Ca	7.7 x 50	8	8	Ca^{2+}	PL1170-1810
Hi-Plex Ca (Duo)	7.7 x 50	8	8	Ca^{2+}	PL1170-1850
Hi-Plex Pb	7.7 x 50	8	8	Pb^{2+}	PL1170-1820
Hi-Plex K	7.7 x 50	8	8	K^{+}	PL1170-1860
Hi-Plex H	7.7 x 50	8	8	H^{+}	PL1170-1830
Hi-Plex Na	7.7 x 50	10	4	Na^{+}	PL1171-1140
Hi-Plex Na (Octo)	7.5 x 50	8	8	Na^{+}	PL1170-1840

Hi-Plex Guard Cartridges, 2/pk

Description	Size (mm)	Particle Size (µm)	Crosslink Content (%)	Counter Ion	Part No.
Hi-Plex Ca	3.0 x 5.0	8	8	Ca^{2+}	PL1670-0810
Hi-Plex Ca (Duo)	3.0 x 5.0	8	8	Ca^{2+}	PL1670-0850
Hi-Plex Pb	3.0 x 5.0	8	8	Pb^{2+}	PL1670-0820
Hi-Plex K	3.0 x 5.0	8	8	K^{+}	PL1670-0860
Hi-Plex H	3.0 x 5.0	8	8	H^{+}	PL1670-0830
Hi-Plex Na	3.0 x 5.0	10	4	Na^{+}	PL1671-0140
Hi-Plex Na (Octo)	3.0 x 5.0	8	8	Na^{+}	PL1670-0840
Guard Cartridge holder for 3.0 x 5.0 mm cartridges					PL1310-0016

Quick Guide to USP Designations for HPLC Columns

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. Rapid Resolution High Throughput (RRHT) columns are now choices in the L1, L7, and L11 categories.

USP Designations

USP Method	USP Packing Materials	Column	Particle Size (µm)	Pore Size (Å)
L1	Octadecyl silane chemically bonded to porous silica or ceramic microparticles, 1.5 to 10 µm in diameter, or a monolithic rod	Poroshell 120 EC-C18	2.7	120
		Poroshell 120 SB-C18	2.7	120
		Poroshell 300SB-C18	5	300
		Poroshell 300 Extend-C18	5	300
		ZORBAX Eclipse Plus C18	1.8, 3.5, 5	95
		ZORBAX Eclipse XDB-C18	1.8, 3.5, 5, 7	80
		ZORBAX StableBond SB-C18	1.8, 3.5, 5, 7	80, 300
		ZORBAX Rx-C18	3.5, 5	80
		ZORBAX Extend-C18	1.8, 3.5, 5, 7	80, 300
		ZORBAX ODS	3, 5, 7	70
		ZORBAX ODS classic	5	70
		Pursuit XRs C18	3, 5, 10	100
		Pursuit C18	3, 5, 10	200
		Pursuit C18-A	3, 5, 10	180
		Polaris C18-Ether	3, 5	200
		SepTech ST60 C18	10	60
		SepTech ST150 C18	10	150
		Agilent Prep C18	5, 10	100
L3	Porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod	ZORBAX HILIC Plus	1.8, 3.5	95
		ZORBAX SIL	5	70
		ZORBAX Rx-SIL	3.5, 5, 7	80, 300
		Pursuit XRs Si	3, 5, 10	100
		Polaris Si-A	5, 10	180
		Agilent Prep	5, 10	100
L7	Octylsilane chemically bonded to totally porous silica particles, 1.5 to 10 µm in diameter, or a monolithic silica rod	Poroshell 120 EC-C8	2.7	120
		Poroshell 120 SB-C8	2.7	120
		Poroshell 300SB-C8	5	300
		ZORBAX Eclipse Plus C8	1.8, 3.5, 5	95
		ZORBAX Eclipse XDB-C8	1.8, 3.5, 5, 7	80
		ZORBAX SB-C8	1.8, 3.5, 5, 7	80, 300
		ZORBAX Rx-C8	1.8, 3.5, 5, 7	80
		ZORBAX C8	5	70
		Pursuit XRs C8	3, 5, 10	100
		Pursuit C8	3, 5, 10	200
		Polaris C8-A	3, 5	180
		Polaris C8-Ether	3, 5	200

(Continued)

USP Designations

USP Method	USP Packing Materials	Column	Particle Size (µm)	Pore Size (Å)
L8	An essentially monomolecular layer of aminopropylsilane chemically bonded to totally porous silica gel support, 3 to 10 µm in diameter	ZORBAX NH2	5	70
		Polaris NH2	5	180
L9	Irregular or spherical, totally porous silica gel having a chemically bonded, strongly acidic cation-exchange coating, 3 to 10 µm in diameter	ZORBAX SCX	5 spherical	300
L10	Nitrile groups chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX CN	5	70
		ZORBAX SB-CN	3.5, 5	80, 300
		ZORBAX Eclipse XDB-CN	3.5, 5	80
L11	Phenyl groups chemically bonded to porous silica particles, 1.5 to 10 µm in diameter	ZORBAX Eclipse XDB Phenyl	5	70
		ZORBAX Eclipse Plus Phenyl-Hexyl	1.8, 3.5, 5	95
		ZORBAX Phenyl	3.5	80
		Poroshell 120 Phenyl-Hexyl	2.7	120
		Pursuit XRs DiPhenyl	3, 5, 10	100
		Pursuit DiPhenyl	3, 5, 10	200
L13	Trimethylsilane chemically bonded to porous silica particles, 3 to 10 µm in diameter	ZORBAX TMS	5	70
L14	Silica gel having a chemically bonded, strongly basic quaternary ammonium anion-exchange coating, 5 to 10 µm in diameter	ZORBAX SAX	5	70
		IonoSpher A	5	120
L17	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the hydrogen form, 7 to 11 µm in diameter	Hi-Plex H	8	N/A
L19	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the calcium form, 9 µm in diameter	Hi-Plex Ca	8	N/A
		Hi-Plex Ca (Duo)	8	N/A
L20	Dihydroxypropane groups chemically bonded to porous silica particles, 3 to 10 µm in diameter	LiChrospher Diol	5	N/A

(Continued)

USP Designations

USP Method	USP Packing Materials	Column	Particle Size (µm)	Pore Size (Å)
L21	A rigid spherical styrenedivinylbenzene copolymer, 5 to 10 µm in diameter	PLRP-S	3, 5, 8, 10, 10-15, 15-20, 50	100
		PLRP-S	3, 5, 8, 10, 10-15, 15-20, 50	300
		PLRP-S	5, 8, 10, 30, 50	1000
		PLRP-S	5, 8, 10, 30, 50	4000
		PLgel	3, 5, 10, 20	50, 100, 500, 10^3, 10^5, 10^5, 10^6, MIXED
L22	A cation-exchange resin made of porous polystyrene gel with sulfonic acid groups, about 10 µm in size	Hi-Plex H	8	N/A
L25	Packing having the capacity to separate compounds with a MW range from 1,000 to 5,000 da (as determined by the polyethylene oxide), applied to neutral, anionic and cationic water-soluble polymers. A polymethacrylate resin base, crosslinked with polyhydroxylated ether (surface contained some residual carboxyl functional gruops) was found suitable	PL aquagel-OH	5, 8	30
L33	Packing having the capacity to separate dextrons by molecular size over a range of 4,000 to 500,000 da. It is spherical, silica-based, and processed to provide pH stability	ZORBAX GF-250	4	150
		Bio SEC-3	3	100, 150, 300
		Bio SEC-5	5	100, 150, 300, 500, 1000, 2000
		ProSEC	5	300

(Continued)

USP Designations

USP Method	USP Packing Materials	Column	Particle Size (µm)	Pore Size (Å)
L34	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the lead form, about 9 µm in diameter	Hi-Plex Pb	8	N/A
L35	A zirconium-stabilized spherical silica packing with a hydrophilic (diol-type) molecular monolayer bonded phase having a pore size of 150Å	ZORBAX GF-250 ZORBAX GF-450	4 6	150, 300
L43	Pentafluorophenyl groups chemically bonded to silica particles by a propyl spacer, 5 to 10 µm in diameter	Pursuit PFP	3, 5	200
L45	Beta cyclodextrin bonded to porous silica particles, 5 to 10 µm in diameter	ChiraDex Chiral	5	100
L50	Multifunction resin with reversed-phase retention and strong anion-exhange functionalities. The resin consists of ethylvinylbenzene, 55% cross-linked with divinylbenzene copolymer, 3 to 15 µm in diameter, and a surface area of not less than 350 m^2 per g. Substrate is coated with quarternary ammonium functionalized latex particles consisting of styrene cross-linked with divinylbenzene	ZORBAX 300SCX	5	300
L52	Weak cation-exchange resin made of porous silica with sulfopropyl groups, 5 to 10 µm in diameter	IonoSpher C	5	120
L53	Weak cation-exchange resin consisting of ethylvinylbenzene, 55% crosslinked with divinylbenzene copolymer, 3 to 15 µm diameter. Substrate is surface grafted with carboxylic acid and/or phosphoric acid functionalized monomers. Capacity not less than 400 µEq/column	Bio SAX	3, 5, 10	300
L56	Propyl silane chemically bonded to totally porous silica particles, 3 to 10 µm in diameter	ZORBAX SB-C3	3, 5	80
L57	A chiral-recognition protein, ovomucoid, chemically bonded to silica particles, about 5 µm in diameter, with a pore size of 120Å	Ultron ES-OVM	5	120
L58	Strong cation-exchange resin consisting of sulfonated cross-linked styrene-divinylbenzene copolymer in the sodium form, about 6 to 30 µm in diameter	Hi-Plex Na Hi-Plex Na (Octo)	10 8	N/A N/A
L60	Spherical, porous silica gel, 10 µm in diameter, the surface has been covalently modified with alkyl amide groups and endcapped	ZORBAX Bonus-RP Poroshell 120 Bonus-RP Polaris Amide-C18	1.8, 3.5, 5 2.7 3, 5	80 120 180

Oligo Solutions

StratoSpheres DNA Cartridges

StratoSpheres DNA Cartridges

- Greater yields of full length products than controlled-pore glass
- Inert support prevents side reactions and improves quality of the end product
- 1000Å pore size permits synthesis of longer oligonucleotide sequences, up to 70-mer
- Certificate of Analysis offered for every batch

StratoSpheres DNA Synthesis Cartridges make it easy to obtain high-quality synthetic DNA oligonucleotides. The high-yielding polystyrene packing delivers more full-length product than conventional controlled-pore glass supports. In addition, the hydrophobic nature of the polystyrene promotes coupling and minimizes non-specific binding to maximize production efficiency. These high-throughput cartridges deliver very economical oligonucleotide synthesis, and provide the high performance expected from macroporous polystyrene supports. StratoSpheres DNA synthesis cartridges deliver maximum flexibility in high-throughput environments.

StratoSpheres DNA Cartridges

Description	Size (nmol)	Part No.
StratoSpheres DNA DMT bz dA	40	PL3554-1602dAbz
	200	PL3554-4602dAbz
StratoSpheres DNA DMT bz dC	40	PL3554-1602dCbz
	200	PL3554-4602dCbz
StratoSpheres DNA DMT ac dC	40	PL3554-1602dCac
	200	PL3554-4602dCac
StratoSpheres DNA DMT ibu dG	40	PL3554-1602dGibu
	200	PL3554-4602dGibu
StratoSpheres DNA DMT dmf dG	40	PL3554-1602dGdmf
	200	PL3554-4602dGdmf
StratoSpheres DNA DMT dT	40	PL3554-1602dT
	200	PL3554-4602dT

TOP, TOP-DNA and TOP-RNA Cartridges

TOP, TOP-DNA and TOP-RNA Cartridges

- Superior yield and purity come from proprietary polymeric resins and optimized buffers
- Typical yield is more than 85% and typical purity is over 90%, eliminating the need for multiple sample-loading steps
- Agilent TOP cartridges use up to two thirds less reagent than products from other vendors

TOP, TOP-DNA and TOP-RNA cartridges provide a high-throughput, simple, cost-effective solution for DNA and RNA oligonucleotide purification. The TOP product range incorporates a unique 96-well plate with removable tubes, streamlined gravity flow or vacuum procedure, and proprietary polymeric resin. Agilent's innovative technology delivers superior yield and purity for standard oligos up to 1 µmol synthesis scale and up to 150-mer in length. Flexibility is assured from a choice of simple gravity flow (for walk-away and low initial setup cost) or vacuum procedure (for fast turnaround – less than 15 minutes for the entire purification process). Up to 10 minutes drying time between each step is permissible with no effect on purification results (drying time after the acetonitrile conditioning step should be kept to a minimum).

TOP and TOP-DNA Cartridges

- Fast throughput improves production efficiency
- Pre-HPLC "sample prep" ability maximizes utility
- Gravity (TOP) or vacuum flow (TOP-DNA) ensures flexibility

TOP-DNA is a high-throughput, simple, fast, cost-effective solution that purifies oligos up to 150-mer in length. Its high binding capacity can purify DNA oligos from 200 nmol to 1 µmol synthesis scales. TOP-DNA can also be used for sample preparation before HPLC purification for very high quality oligos in large-scale analysis. The proprietary polymeric resin is compatible with direct loading of AMA deprotected oligo solutions.

TIPS & TOOLS

For more information on TOP RNA, view this Application Note on-line: High Performance RNA oligonucleotide purification using Agilent TOP-RNA (publication # 5990-8974EN), **www.agilent.com/chem/library**

TOP-RNA Cartridges

- A complete solution for RNA oligo purification to enhance productivity
- High throughput and automation friendly, freeing up operator time
- Less reagent use reduces operating costs

With TOP-RNA you can purify short and long RNA oligos, siRNA to 21-mer and long RNA to 60-80-mer. The high binding capacity purifies RNA oligos up to 1 µmol. The proprietary polymeric resin and validated protocol allow deprotection of 2'hydroxyl group without removal of the 5'trityl group.

TOP, TOP-DNA and TOP-RNA Cartridges

Description	Sorbent Mass (mg)	Volume (mL)	Unit	Part No.
TOP-RNA well plate tubes for 1 µmol scale	100	1.8	96/pk	7573915C
TOP-RNA well plate tubes for 1 µmol scale	100	1.8	20 x 96/pk	7573915B
TOP-DNA well plate tubes for 1 µmol scale	150	1.8	96/pk	7572915C
TOP cartridge	500	6	30/pk	12102301
TOP cartridge	300	6	30/pk	12102300
Mega Bond Elut TOP	3 g	20	20/pk	14251921
TOP-DNA well plate tubes for 1 µmol scale	150	1.8	20 x 96/pk	7572915B
TOP well plate tubes for 50 nmol scale	25	1.8	96/pk	75719025
TOP well plate tubes for 200 nmol scale	50	1.8	96/pk	75719050
TOP well plate tubes for 200 nmol scale, high capacity	100	1.8	96/pk	7571901C
96-well plate sealing mat			50/pk	5133005
Disposable waste tray			25/pk	5133001
TOP reusable base plate				75400001
VersPlate Base Plate			100/pk	75700001

Table of Contents

LC and LC/MS Columns for Biomolecules

From sample purification to analysis, Agilent's biomolecule columns and supplies are easy to integrate into your workflow for a complete, reproducible, and high-quality solution.

In this section of the catalog you will also find advice and tips on solvent choice, mobile phase modification, optimization, and example separations to assist you in column selection and method development.

Agilent has complete solutions for your needs. These include the Agilent 1260 Infinity Bio-inert LC system with a metal-free sample path and the Agilent 1290 Infinity LC, designed to provide highest speed, resolution, and ultra-sensitivity for UHPLC applications, including those utilizing Agilent wide-pore 300Å ZORBAX StableBond columns. Biomolecules may be complex in structure, but their analysis is simplified by using Agilent HPLC columns, systems, and supplies.

What is a biomolecule?

Biomolecules are compounds made by living organisms. They can range in size from amino acids and small lipids to large polynucleotides such as DNA or RNA.

In this section, we deal with the separation of:

Proteins – separation based on size with size exclusion chromatography, charge with ion-exchange chromatography, and hydrophobicity with reversed-phase chromatography.

Peptides – biocolumns for the analysis and purification of the full range of peptides, including hydrophobic, hydrophilic, basic and acidic peptides across the full size range. Also, columns for peptide mapping by HPLC and UHPLC.

DNA/RNA oligonucleotides – reversed-phase and ion-exchange options for DNA and RNA oligos, and with particle pore sizes to cover the full range of oligonucleotide sizes, from small synthetic oligos to large plasmids.

Amino acids – the ZORBAX Eclipse Amino Acid Analysis HPLC columns provide a high efficiency solution for rapid analysis of 24 amino acids. Typical analysis times range from 14 minutes, with a 75 mm column, to 24 minutes with a 150 mm column.

Broad-distribution polymers – analysis of lipids, polysaccharides and drug delivery compounds using polymeric columns and standards to determine their molecular weight distribution and composition. These compounds tend to exhibit broad MW distributions, in contrast to other biomolecules that have narrow MW distributions or a defined molecular weight.

What is a biocolumn?

Biochromatography columns, or biocolumns, are liquid chromatography columns used for the separation of biological compounds such as peptides and proteins, oligonucleotides and polynucleotides, and other biomolecules and complexes. Biocolumns are specifically designed for biomolecule analysis with larger pore sizes to accommodate the larger molecule sizes. Media is designed to minimize non-specific binding of analytes for improved recovery. Separation mechanisms are chosen to either retain biological function so bioactivity is not lost during analysis, or to deliberately denature for primary structure characterization.

Typically, HPLC has been used to separate biomolecules. Now, advanced techniques such as UHPLC are becoming a popular choice because multiple separation mechanisms are needed in the characterization of biomolecules. Therefore, Agilent offers advanced chemistries developed specifically for the separation of biomolecules using size exclusion, reversed-phase, ion-exchange, and affinity functionalities, all of which are covered in this section of the catalog.

Protein Separations

Proteins are complex molecules that require multiple techniques to provide full characterization. They exist as three-dimensional structures and it is this structure that confers their biological activity.

The sequence of the amino acid chains defines the primary structure of the protein. Hydrogen bonding between amino acids of the primary structure then confers a secondary structure typically in the form of alpha helices and pleated sheets. A further series of interactions, hydrogen bonding, ionic, hydrophobic and disulphide bridges, between regions of the secondary structure, then provides the tertiary protein structure, or three-dimensional conformation. If the protein is composed of a number of amino acid chains, the interaction between these chains gives the quaternary structure.

When looking at methods for protein characterization, it is therefore clear from Figure 1 that techniques will be required that characterize the protein in its native state, without disrupting the tertiary and quaternary structures. We also need techniques for assessing the primary amino acid sequence, in the fully denatured state with the three-dimensional structure stripped away.

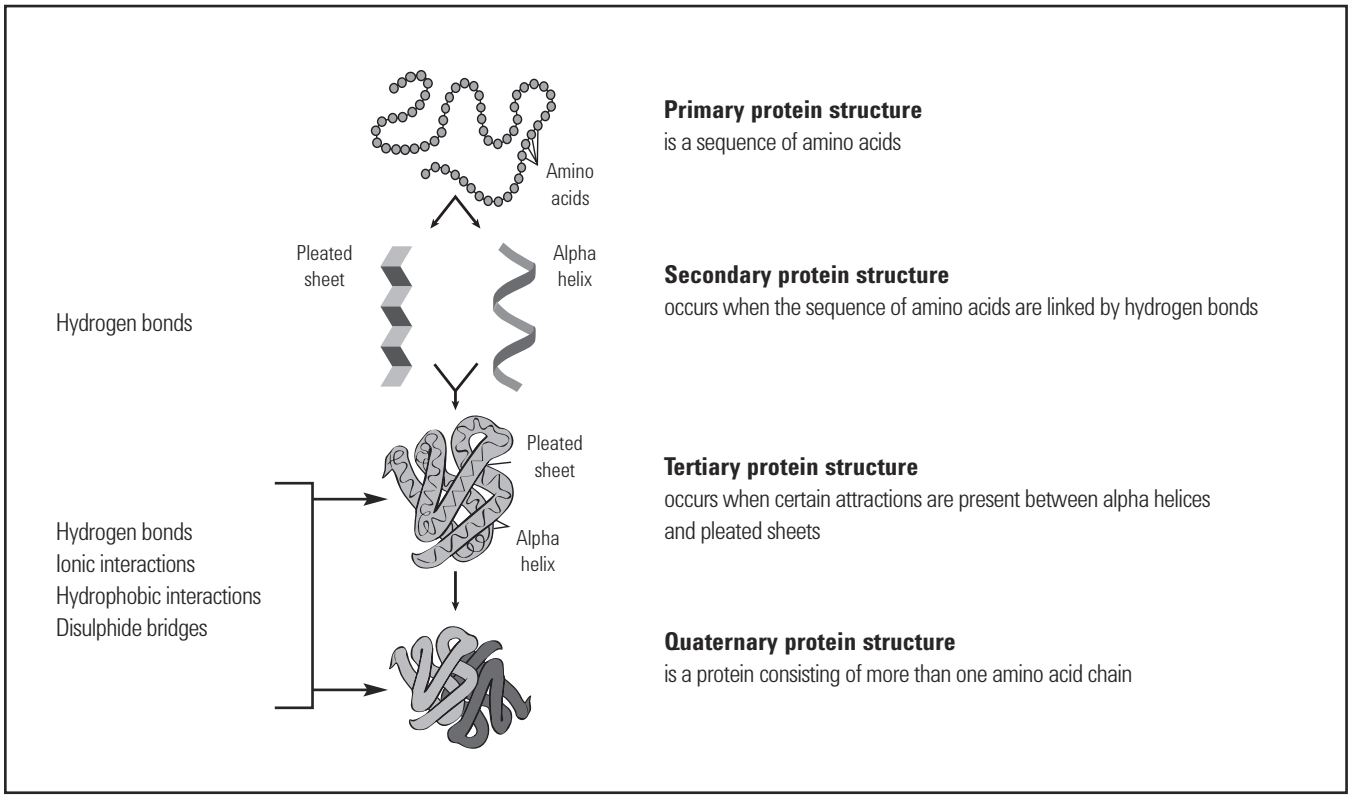


Figure 1. Schematic showing the various levels of protein structure.

The environment of the protein can influence, stabilize, or disrupt the structure of the protein. Factors to consider include pH, temperature, salt concentrations, aqueous or organic solvent content, and for some proteins, the presence of a stabilizing small molecule or metal ion. Protein structure can also be disrupted by the use of sulfhydryl reducing agents to break -S-S- bonds or chaotropic agent, like urea or guanidine HCl. With the complexity of proteins and the intramolecular interactions that determine the three-dimensional structure, you can also expect that there will be intermolecular associations between protein molecules and other molecular entities and the surfaces with which they come into contact. This can result in protein complexes, aggregation (with possible precipitation), and deposition on surfaces, including those of the HPLC column and system. Therefore, you should consider the handling and environment in which the protein is maintained.

Protein Column Selection Guide

Application	Technique	Agilent Columns	Notes
Primary structure analysis	UHPLC/HPLC reversed-phase separations	ZORBAX 300SB Poroshell 300SB PLRP-S	Reversed-phase separations require (or cause) denaturing of the protein to obtain detailed information about the amino acid sequence and/or amino acid modifications (including post-translational modifications).
Aggregation analysis	Size exclusion separations	Bio SEC-3 Bio SEC-5 ProSEC 300S ZORBAX GF	Aggregates in protein biopharmaceuticals are of major concern as they can induce an immunogenic response and can influence the composition of the final formulation.
Charge variant analysis	Ion-exchange separations	Agilent Bio IEX Agilent Bio MAb PL-SAX PL-SCX	The ratio of individual amino acids determines the net charge of the protein molecule. The pH at which the net charge is zero is called the isoelectric point (pI). When the solution pH is less than the pI, the protein will be positively charged (acidic), and when the solution pH is greater than the pI, the protein is negatively charged (basic). For ion-exchange analysis, we recommend the eluent pH be at least one pH unit away from its pI. Protein analysis using ion-exchange columns requires buffered mobile phase and either salt gradients or pH gradients for elution.

Higher resolution of oxidation study

Column: **ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**

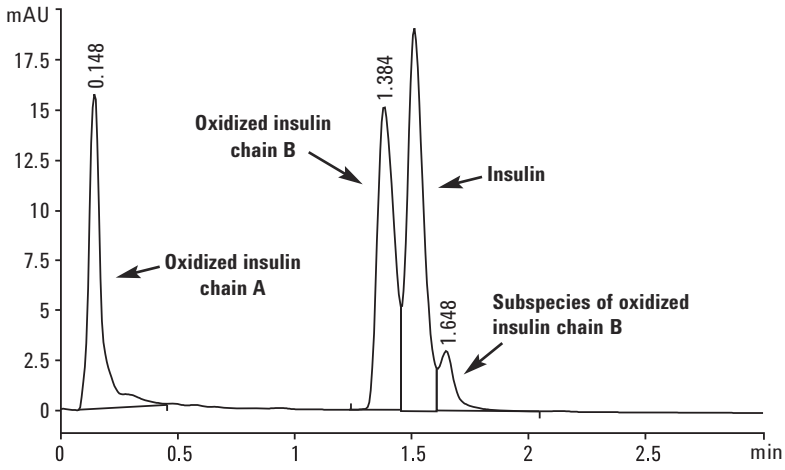
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

Flow Rate: 1.0 mL/min

Gradient: 33 to 50% B, 0 to 4 min

Detector: 1290 Infinity LC with diode array detector at 280 nm

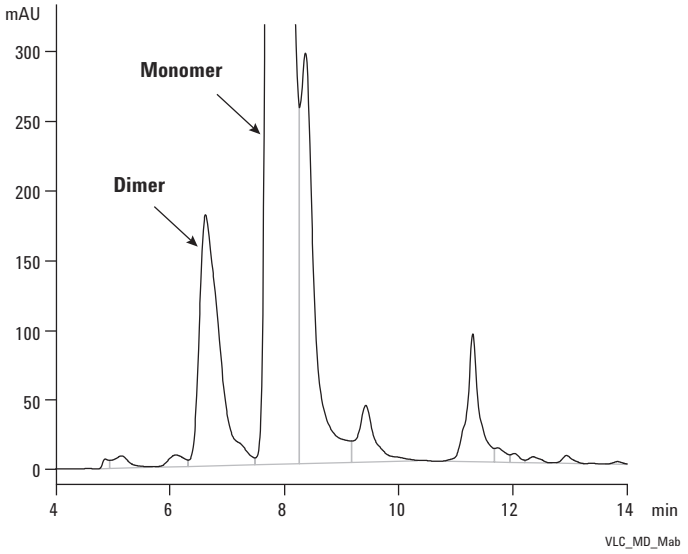
Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)



It is evident that the oxidized insulin chains are resolved from insulin in under 2 minutes using the Agilent ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8 µm column.

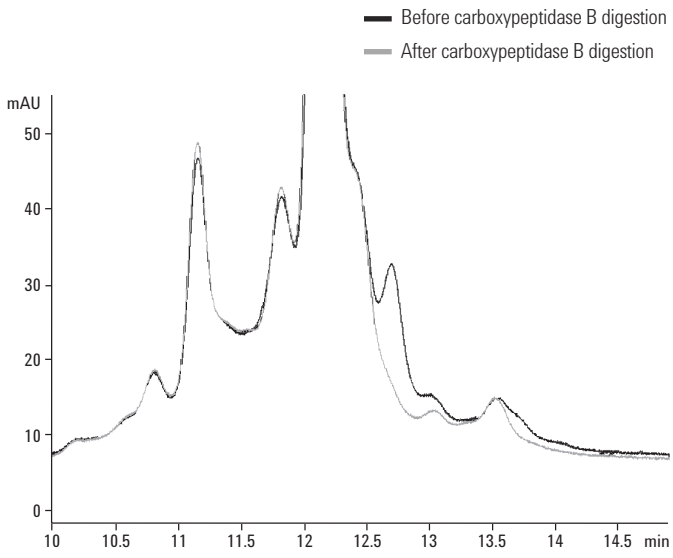
Intact MAb monomer and dimer separation

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM
Isocratic:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – intact
Injection:	5 µL
Detector:	UV 220 nm
Temperature:	Ambient



Separation of charge variants of human IgG1 with pH gradient

Column:	**Agilent Bio MAb 5190-2411 2.1 x 250 mm, 5 µm**
Mobile Phase:	A: 10 mM Na_2HPO_4, pH 6.0 B: A + 0.5 M NaCl or just 0.5 M Na_2HPO_4, pH 6.0
Flow Rate:	2 mL/min
Gradient:	0.5 min hold with mobile phase A followed by a linear gradient to 45% B in 15 min (elapsed time 15.5 min); then 60% B at 15.6 min continued to 20 min. Column flushed with 100% B for 15 min before re-equilibration for the next run. pH Gradient: A: 5 mM Na_2HPO_4, buffer pH 5.5 and B: 40 mM NA_2HPO_4 (not buffered, pH 8.9). 2% B/min at 1 mL/min for 15 min, followed by a column wash with 90% B for 5 min.
Detector:	UV at 220 nm
Sample:	One mg each/mL in mobile phase A Monoclonal antibodies (MAb) -human IgG1 (5 mg/mL stock solution) derived from CHO cells
Instrument:	Agilent 1200 SL system with diode array detector



MAb c-terminal cleavage: Human IgG1 MAb, 1 mg/mL in 25 mM Na_2HPO_4 buffer, pH 7.5, was incubated with approximately 25 units of the carboxypeptidase B for 18 hours and 10 µL samples were injected.

Peptide Separations

Peptide Mapping

Peptide mapping is required for the characterization of proteins. It is used to confirm the identity of a protein and to identify and quantify post-translational modifications.

The purified protein is first digested using an enzyme, such as trypsin, yielding a range of peptide fragments. The specificity of the enzyme cleavage produces a fingerprint of peptides which is characteristic of that protein. Identification of the peptide fragments confirms the identity of the protein, and changes in the profile of the peptide digest can be used to identify post-translational modifications to that protein that may have occurred during the manufacturing or purification processes.

Reversed-phase UHPLC/HPLC is the preferred technique for the analysis of peptide digests with either MS or UV detection. LC/MS is used for the identification of the peptide fragments and determination of sequence coverage whereas LC/UV is more commonly used for peptide map comparisons in the monitoring/QC segments. To achieve sufficient resolution for quantification and identification, longer column lengths or higher efficiency particles such as the sub-2 µm ZORBAX RRHD, or superficially porous Poroshell are recommended.

Peptide digests are complex mixtures, and for complete coverage, i.e. resolution of the individual peptides, a high efficiency/high resolution column is required. The peptide fragments can range in size and hydrophobicity, so Agilent offers several columns for peptide mapping. There are three options: pore sizes, particle sizes, and superficially porous and fully porous for UHPLC separations.

TIPS & TOOLS

Capillary electrophoresis is an alternative technique to liquid chromatography for the separation of complex peptide mixtures. Further information can be found in the following Case Study:

An orthogonal view of peptide mapping – analysis of bovine serum albumin digest using capillary electrophoresis and quadrupole time-of-flight mass spectrometry (publication # 5990-7631EN)

www.agilent.com/chem/library

Peptide Mapping Column Selection

Recommended column choices determined by system/column pressure maximum and peptide size/hydrophobicity.

Application	Technique	Agilent Columns	Notes
Large peptide fragments/hydrophobic peptide core	400 bar HPLC	Poroshell 300 SB-C18 ZORBAX 300SB-C18, 3.5 µm	Agilent 1200 Infinity LC
	600 bar UHPLC	Poroshell 300 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	ZORBAX RRHD 300SB-C18, 1.8 µm Poroshell 300 SB-C18	Agilent 1290 Infinity LC
Small hydrophobic peptides	400 bar HPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1200 Infinity LC
	600 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1290 Infinity LC

If you have an Agilent 1290 Infinity LC in your lab, we recommend starting with a ZORBAX RRHD 300SB-C18 column to screen your peptide map.

Increased resolution for peptide mapping

Column: **ZORBAX 300SB-C18**
858750-902
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

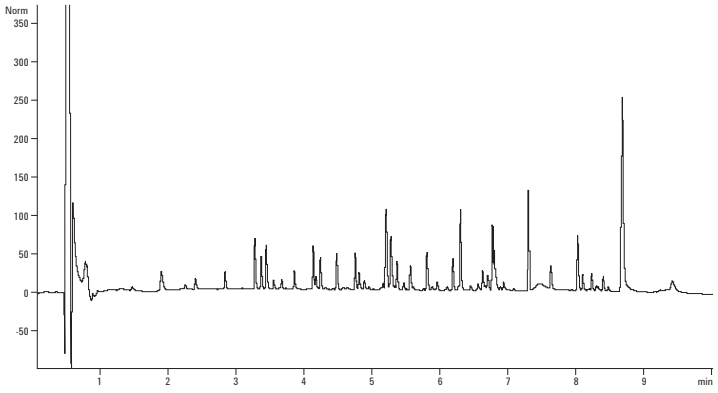
Flow Rate: 0.5 mL/min

Gradient: 2% B for 1 min, 2 to 45% B for 8.8 min, 45 to 95% B for 0.2 min, 95% B for 2 min, 95 to 2% B for 0.2 min

Temperature: 50 °C

Detector: 1290 Infinity LC with diode array detector at 280 nm

Sample: Enzymatic protein digest (MAb)



The longer 100 mm Agilent ZORBAX RRHD 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is under fifteen minutes.

Separation of Natural and Synthetic Peptides

Purification columns and media are required for the isolation and analysis of natural and synthetic peptides. Purity and recovery determination of the isolated or purified peptide requires the use of high efficiency columns. The primary technique used for the isolation and purification, and analysis, is reversed-phase HPLC.

The fractions from a purification or isolation workflow and the final peptide product are analyzed for purity using high efficiency columns. The peptides will vary in size, charge and hydrophobicity and so, as with peptide mapping applications, Agilent offers a range of columns to provide optimum separations of the full range of peptides. For small peptides, typically less than 10 amino acid residues, the smaller pore UHPLC materials are used, but if the peptide is larger, contains more amino acid residues, or exists in a dimeric or multimeric form, then the larger pore size 300Å columns provide better separations due to improved mass transfer.

Natural and Synthetic Peptides Column Selection

Recommended column choices as determined by system/column pressure maximum for the analysis of natural and synthetic peptides.

Application	Technique	Agilent Columns	Notes
Larger peptides with more than 10 amino acid residues	400 bar HPLC	Poroshell 300 SB-C18 ZORBAX 300SB-C18, 3.5 µm PLRP-S	Agilent 1200 Infinity LC
	600 bar UHPLC	Poroshell 300 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC
	1200 bar UHPLC	ZORBAX RRHD 300SB-C18, 1.8 µm	Agilent 1290 Infinity LC
Peptides with typically less than 10 amino acid residues	400 bar HPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18 PLRP-S	Agilent 1200 Infinity LC
	600 bar UHPLC	Poroshell 120 EC-C18 Poroshell 120 SB-C18	Agilent 1260 Infinity LC and 1260 Infinity Bio-inert Quaternary LC

Reversed-phase columns are also the first choice for purifying large numbers of individual peptides or larger amounts of a particular peptide. High efficiency, small particle pre-packed prep columns are available for the high efficiency purification of small amounts of peptides, and larger particle columns and bulk media for the larger scale purifications, as shown in Table 1.

Table 1. Agilent columns for small- to large-scale peptide purifications.

	Amount of Peptide Required		
Agilent Column	**mg**	**g**	**kg**
ZORBAX Prep HT 300StableBond	→		
VariTide RPC	→	→	
PLRP-S	→	→	→

After solid phase synthesis (SPS) using a polystyrene resin such as one of the Agilent StratoSpheres products, the peptide is cleaved from the support and the resultant mixture is separated to obtain the target peptide. A high efficiency column is needed for the purification as the candidate peptide must be resolved from peptides that are very similar in structure. Check **www.agilent.com** for further information.

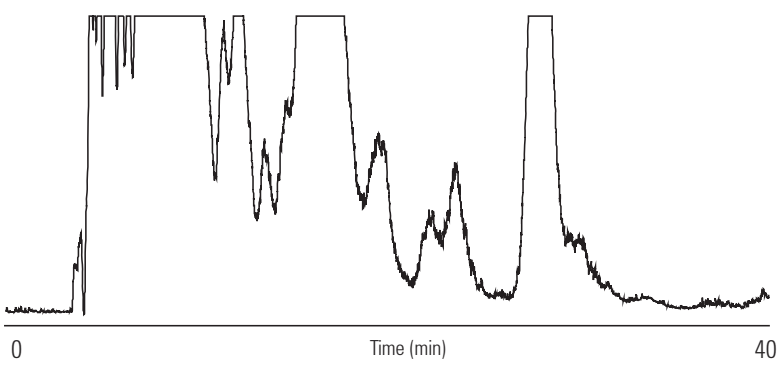
Preparative scale purification of Leuprolide by concentration overload

Column: **PLRP-S 100Å, 10 µm PL1412-4100**

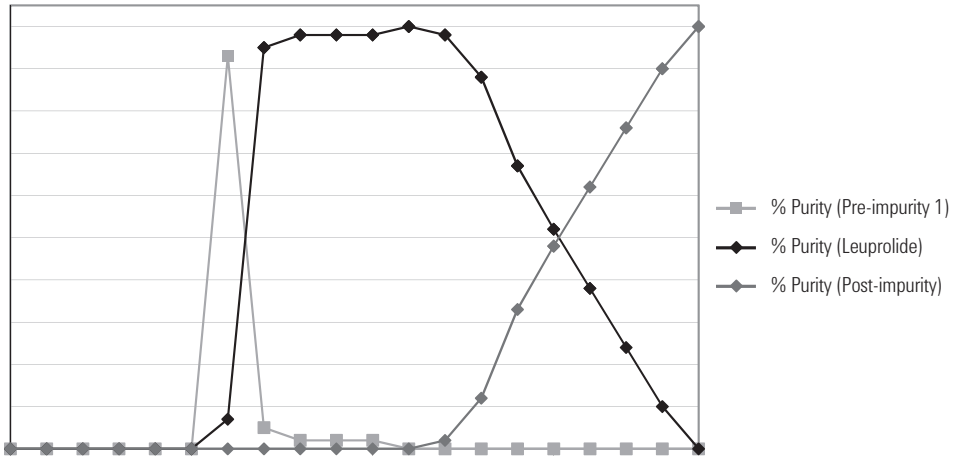
Bulk Media: **Load & Lock 4001 Column PCG93LL500X25**

Mobile Phase: Isocratic separation using 0.1% TFA in 28% ACN:72% water

Flow Rate: Linear velocity 360 cm/hr



Crude leuprolide separation of 30 mg on-column load.



Fraction analysis – the concentration overload purification.

DNA and RNA Oligonucleotide Separations

There is a renewed interest in oligonucleotides (oligos) as they are used in more and more applications, including potential therapeutics. The synthesis workflow is similar to that used for the more established synthetic peptide production, i.e. an activated solid phase synthesis resin is used with sequential addition of specific nucleotides to build the desired sequence.

The nucleotide building blocks are protected at the 5′ hydroxyl end with a dimethoxytrityl (DMT) group and the cleaved target oligo will have this protected group still attached. As DMT is hydrophobic, it is a useful handle that can be used for the first stage step. To increase the stability of the oligonucleotide, particularly to enzyme degradation, it may be chemically modified, for example by replacing oxygen with sulfur to produce phosphorothioates.

When using chemical synthesis to produce biomolecules, the coupling efficiency of each additional cycle is never 100%. The sample, after cleavage from the solid phase synthesis support, will contain deletion sequences, oligos where one or more residues are missing, and some amount of larger oligos produced by double coupling or branching. The sample mixture is complex and high efficiency techniques are required for analysis.

There are three UHPLC/HPLC techniques that are routinely used for oligonucleotide separations:

Trityl-on: This procedure is relatively simple to perform and separates the full-length target oligo, which still has the DMT group attached, from the deprotected failure sequences. The analytical information obtained is limited and this is generally considered to be a purification method.

Ion-exchange separations of the trityl-off, deprotected oligos: This method uses the negative charge on the backbone of the oligo to facilitate the separation. Resolution is good for the shorter oligos but decreases with increasing chain length. Aqueous eluents are used but oligos are highly charged, and high concentrations of salt are needed to achieve elution from the column.

Ion-pair reversed-phase separation of the trityl-off, deprotected oligos: This technique uses organic solvents and volatile ion-pairing agents and is suitable for LC/MS. The technique is best performed with high efficiency particles. Conditions that fully denature the oligos and prevent association with complimentary sequences are required. Thus, the separation is best performed at elevated temperatures.

DNA and RNA Oligonucleotide Column Selection

Application	Technique	Agilent Columns	Notes
Trityl-on/trityl-off oligonucleotides	Trityl-on	PLRP-S 50 µm media	Separates due to differences in hydrophobicity. Ideal for the separation of trityl-on from trityl-off oligos and is also used for ion-pair reversed-phase separations of deprotected oligos.
Deprotected oligonucleotides	Ion-pair reversed-phase separation of the trityl-off, deprotected oligos	PLRP-S 3 µm to 50 µm	
Deprotected oligonucleotides	Ion-exchange separations of the trityl-off, deprotected oligos	PL-SAX 1000Å	Separates deprotected oligos under denaturing high pH conditions. The quaternary amine functionality on the polymeric particles enables ion-exchange separations at high pH, improving chromatography for self-complementary sequences.

TIPS & TOOLS

Further information can be found in the following publications:
Agilent PLRP-S 100Å HPLC Columns and Media (publication # 5990-8187EN)
Agilent PL-SAX 1000Å HPLC Columns and Media (publication # 5990-8200EN)
www.agilent.com/chem/library

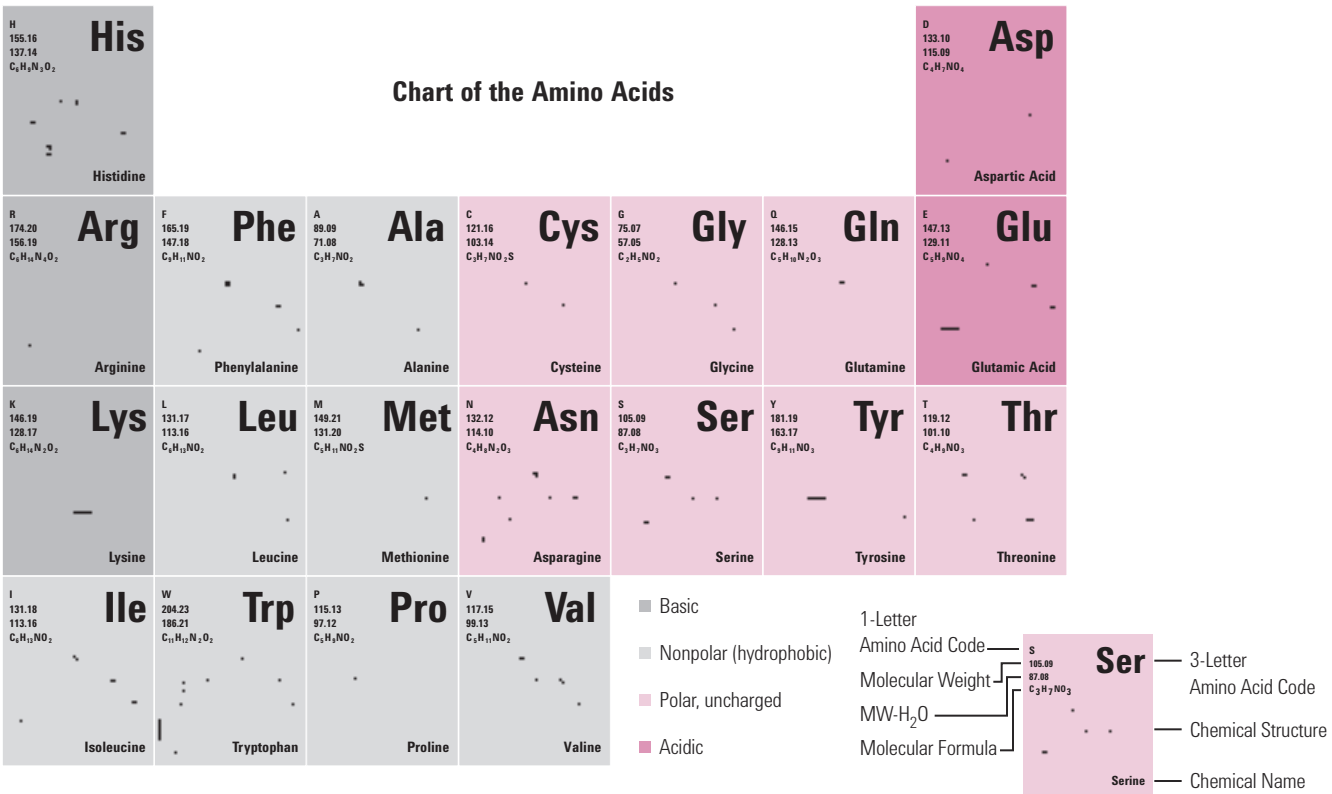
Amino Acid Analysis

Agilent offers several good options for separation of amino acids, including the Agilent ZORBAX Eclipse AAA column which uses an updated protocol and is specially tested using amino acids. 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 columns and 24 min (18 min analysis time) on the 15 cm column. Exceptional sensitivity (5 to 50 pmol with diode array or fluorescence detectors) and reliability are achieved using both OPA- and FMOC-derivatization chemistries in one fully automated procedure using the Agilent 1200 Infinity LC. The newer ZORBAX Eclipse Plus C18 column is also an excellent choice for amino acid separations.

ZORBAX Eclipse AAA Column Selection

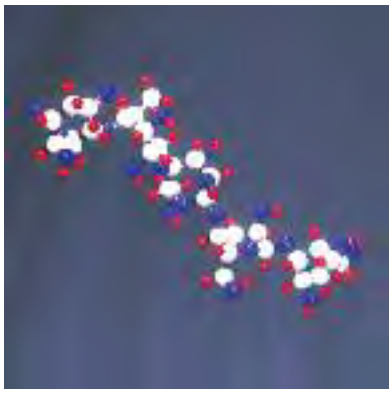
Application	Diameter x Length (mm)	Particle Size (µm)
Analytical routine sensitivity	4.6 x 150	5.0
Analytical routine sensitivity, high-resolution using FLD	4.6 x 150	3.5
Analytical routine sensitivity, high-throughput	4.6 x 75	3.5
Solvent Saver high sensitivity, high-resolution	3.0 x 150	3.5

For more information on the ZORBAX Eclipse Plus C18 column, turn to page 248.



Broad Distribution Biomolecules

Carbohydrates, Lipids and PEGs



Aqueous size exclusion chromatography employing columns packed with polymeric media can be extremely useful when investigating biomolecules and their derived species with broad molecular weight distributions. Examples include PEGylated proteins and complex polysaccharides which find use in biopharma applications. The wide pore size distribution of polymeric SEC columns compared to silica-based material are excellent for samples with polydispersities greater than one.

Broad Distribution Biomolecule Column Selection		
Low MW polymers and oligomers, oligosaccharides, PEGs, lignosulfonates	2 or 3 PL aquagel-OH columns • PL aquagel-OH 8 µm • PL aquagel-OH 20 5 µm • PL aquagel-OH MIXED-M 8 µm	The PL aquagel-OH analytical series has a pH range of 2-10, compatible with organic solvents (up to 50% methanol), mechanical stability up to 140 bar (2030 psi) and low column operating pressures.
Polydisperse biopolymers, polysaccharides, cellulose derivatives	2 or 3 PL aquagel-OH columns • PL aquagel-OH MIXED-H 8 µm • PL aquagel-OH 60/50/40 8 µm	
Very high MW polymers, hyaluronic acids, starches, gums	PL aquagel-OH 60/50/40 15 µm in series	

UHPLC/HPLC Techniques

High-performance liquid chromatography, HPLC, is a chromatographic technique that can separate a mixture of compounds and is used in biochemistry and analytical chemistry to identify, quantify and purify the individual components of the mixture. There has been an evolution toward ultra high-performance liquid chromatography (UHPLC) which is widely accepted for high-efficiency separations of small- to medium-sized molecules, and has been used to reduce analysis time and/or to increase resolution. The use of UHPLC has been extended to large biomolecules with the introduction of wide pore chromatographic media in columns that can withstand pressures of 600 to 1200 bar.

On the following pages you will see the wide range of columns that Agilent offers for the HPLC and UHPLC separation of proteins and other biomolecules.

UHPLC/HPLC Techniques for Biomolecule Analysis

Technique	Advantages	Disadvantages
Reversed-Phase	• High resolution • High capacity • Relatively simple • Sample concentrated on-column • Small particle, 1.8 µm, for UHPLC separations • Polymeric media for unsurpassed chemically and thermally stable	• Denaturing conditions • High efficiency silica columns cannot be cleaned using aggressive solvents when performing purifications
Ion-Exchange	• Good recovery of biological activity • High capacity • Sample concentrated on-column	• Limited MS compatibility due to presence of salts
Size Exclusion	• Good recovery of biological activity • Non-interactive technique with good sample recovery	• No sample concentration • Limited capacity
Affinity	• Highly selective • Good recovery of biological activity • Sample concentrated on-column • Often single step isolation	• No sample concentration • Limited capacity

Reversed-Phase HPLC

Confidently perform high-resolution separations

Reversed-phase UHPLC/HPLC separates solutes based on differences in hydrophobicity, with the least hydrophobic peak eluting first. This high-resolution technique is capable of separating peptides, proteins and oligonucleotides that differ by only one amino acid or nucleotide residue.

Because HPLC uses organic solvents (such as acetonitrile, methanol, ethanol and propanol) it is also a denaturing technique that disrupts a biomolecule's three-dimensional structure. This allows you to obtain information about a molecule's primary structure and sequence, as well as variations in the sequence to be identified.

Agilent offers the industry's broadest range of wide-pore reversed-phase columns, all backed by technical support experts and application chemists around the globe. This section features the following column innovations:

- **ZORBAX 300Å pore silica columns** – an industry first for reversed-phase protein and biomolecule separations – are available in 6 phases, along with a broad array of sizes. For fast UHPLC separations, we also offer a 1.8 µm particle size option that withstands pressures up to 1200 bar, and can be used with high-pressure instruments, such as Agilent's 1290 Infinity LC.
- **Agilent Poroshell columns** feature the industry's first solid core/porous shell particle. Our wide-pore Poroshell 300 columns are ideal for fast chromatography, and are available in a variety of phases.
- **Agilent PLRP-S columns** contain polymer particles, and can be used to separate peptides and proteins of various sizes and DNA/macromolecular complexes. These columns are unique in that they are 100% organic, can withstand temperatures as high as 200 °C, and can be used under conditions from pH 1 to pH 14.
- Choose from a range of column sizes, particle sizes (3-8 µm for analytical separations) and pore sizes (100Å to 4000Å). Preparative columns (10-50 µm) are also available, either prepacked in columns or as bulk material.

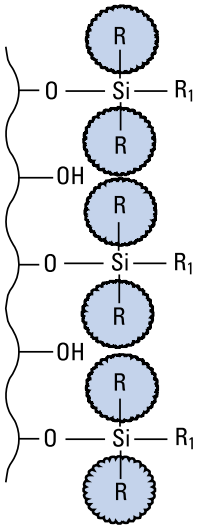
Reversed-Phase Column Selection

Application	Agilent Columns	Notes
Proteins and polypeptides	ZORBAX 300Å, 1.8 µm • RRHD 300SB-C18 • RRHD 300SB-C8 • RRHD 300SB-C3 • RRHD 300-Diphenyl • RRHD 300-HILIC	Improved packing processes achieve stability up to 1200 bar for use with the Agilent 1290 Infinity LC. RRHD 1.8 µm columns are available in 50 and 100 mm lengths for fast or high resolution – truly high definition – separations of the most complex samples.
	ZORBAX 300Å StableBond • 300SB-C18 • 300SB-C8 • 300SB-C3 • 300SB-CN	Wide-pore, 300Å columns are necessary for an efficient separation of proteins and peptides, or other large molecules, to allow these analytes to completely access the bonded phase. C18 and C8 are ideal for complex protein and protein digest separations. StableBond provides enhanced stability for low pH.
	ZORBAX 300Å Extend-C18	Incorporate a unique patented bidentate silane, combined with a double-endcapping process that protects the silica from dissolution at high pH – up to pH 11.5.
Peptides and proteins up to 1,000 kDA, monoclonal antibodies and intact proteins	Poroshell 300 • 300SB-C18 • 300SB-C8 • 300SB-C3 • 300Extend-C18	Poroshell columns use a unique particle made with a 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.
Small hydrophilic peptides in protein digests	Poroshell 120	The 120Å pore size is ideal for the fast high resolution analysis of small hydrophilic peptides and peptide fragments in protein digests.
Peptides to DNA	PLRP-S • 100Å • 300Å • 1000Å • 4000Å	Particles are inherently hydrophobic so an alkyl ligand bonded phase is not required for reversed-phase separations. This gives a highly reproducible material that is free from silanols and heavy metal ions.
Small molecules/peptides/oligonucleotides	PLRP-S 100Å	
Recombinant peptides/proteins	PLRP-S 300Å	
Large proteins	PLRP-S 1000Å	
DNA/high speed separation	PLRP-S 4000Å	

ZORBAX 300Å StableBond

Agilent ZORBAX 300Å StableBond 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 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 five different bonded phases (C18, C8, C3, CN, and Diphenyl*) 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 °C. 300SB-C18 and 300SB-C8 columns are an ideal choice for complex protein and protein digest separations. These columns are also available in capillary (0.3 and 0.5 mm id) and nano (0.075 and 0.10 mm id) dimensions for reversed-phase LC/MS separations of protein digests. Capillary and nano columns can be used for either 1-D or 2-D proteomics separations.

*Diphenyl is available in a 1.8 µm particle size only.



Sterically Protected 300StableBond Bonded Phase

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%
ZORBAX 300-Diphenyl	300Å	45 m^2/g	80 °C	1.0-8.0	Yes	1.9%

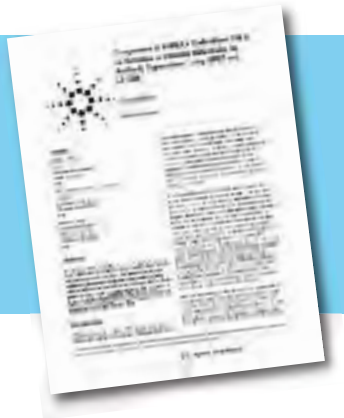
Specifications represent typical values only

*300StableBond 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

Further information can be found in the following publication:

Comparison of ZORBAX StableBond 300Å LC Columns to Optimize Selectivity for Antibody Separations Using HPLC and LC/MS (publication # 5989-6840EN)
www.agilent.com/chem/library



Higher resolution of intact monoclonal antibody

Column: **ZORBAX RRHD 300SB-C8**
857750-906
2.1 x 50 mm, 1.8 µm

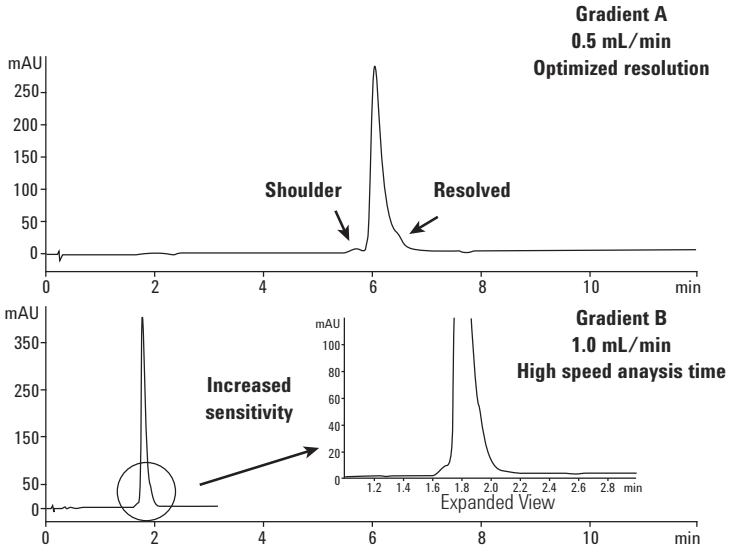
Mobile Phase: A: H_2O:IPA (98:2) + 0.1% TFA (v/v)
B: IPA:ACN:H_2O (70:20:10) + 0.1% TFA (v/v)

Flow Rate: Between 0.5 mL/min and 1.0 mL/min

Gradient: Multi-segmented and linear elution

Temperature: 80 °C

Detector: Agilent 1290 Infinity LC with auto injector (ALS), binary pump and thermostatted oven and diode array detector (DAD), UV, 225 nm



Higher resolution of oxidation study

Column: **ZORBAX RRHD 300SB-C18**
857750-902
2.1 x 50 mm, 1.8 µm

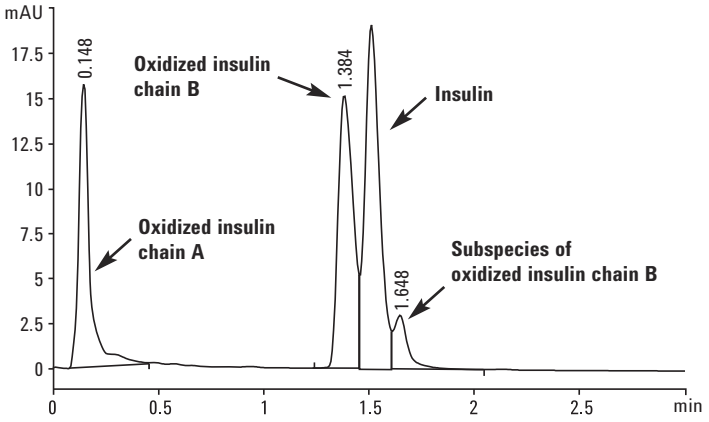
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

Flow Rate: 1.0 mL/min

Gradient: 33 to 50% B, 0 to 4 min

Detector: 1290 Infinity LC with diode array detector at 280 nm

Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)



It is evident that the oxidized insulin chains are resolved from insulin in under 2 minutes using the Agilent ZORBAX RRHD 300SB-C18 2.1 x 50 mm, 1.8 µm column.

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 100-500 kDa.

Improved reproducibility of monoclonal antibodies

Column: **ZORBAX RRHD 300SB-C8**
857750-906
2.1 x 50 mm, 1.8 µm

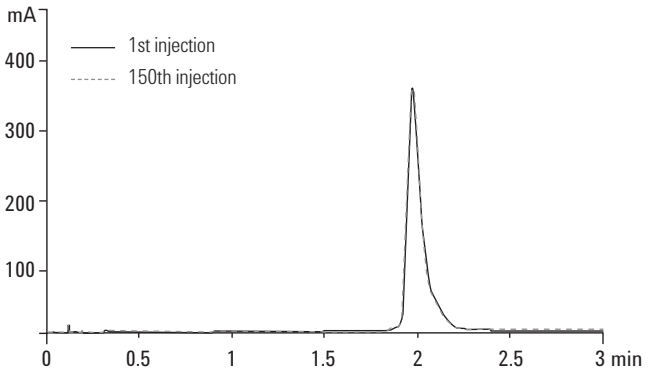
Mobile Phase: A: H_2O:IPA (98.2), 0.1% TFA
B: IPA:ACN:H_2O (70:20:10), 0.1% TFA

Flow Rate: 1.0 mL/min

Temperature: 80 °C

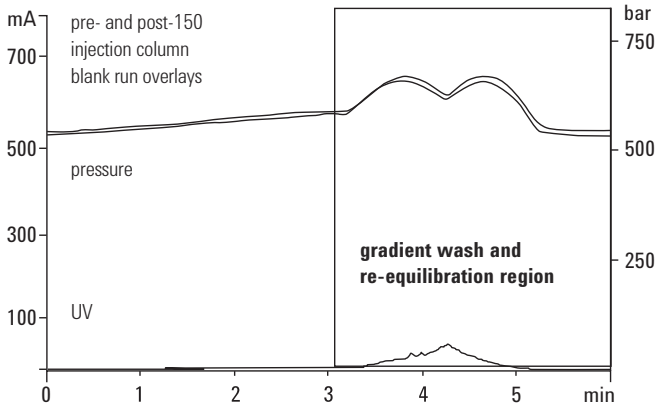
Detector: 1290 Infinity LC with diode array detector at 225 nm

Sample: MAb



Gradient timescale

Time (min)	% Solvent B
0.00	25
3.00	35
4.00	90
5.00	25



Excellent column reproducibility and protein recovery using Agilent ZORBAX 300SB-C8.

Increased resolution for peptide mapping

Column: **ZORBAX 300SB-C18**
858750-902
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

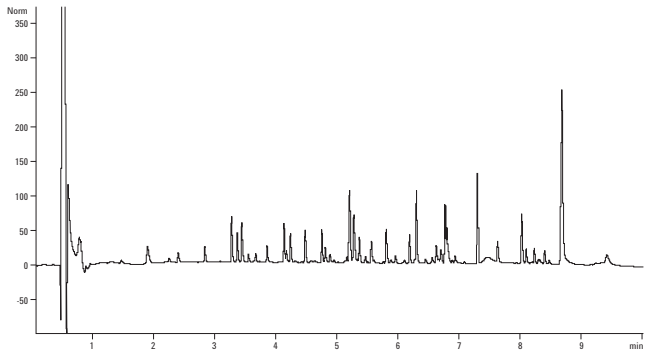
Flow Rate: 0.5 mL/min

Gradient: 2% B for 1 min, 2 to 45% B for 8.8 min, 45 to 95% B for 0.2 min, 95% B for 2 min, 95 to 2% B for 0.2 min

Temperature: 50 °C

Detector: 1290 Infinity LC with diode array detector at 280 nm

Sample: Enzymatic protein digest (MAb)



The longer 100 mm Agilent ZORBAX RRHD 300SB-C18 column provides maximum resolution for protein digests – in this sample the total run time, including washing and equilibration, is under fifteen minutes.

Peptides: Effect of TFA concentration

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

Mobile Phase: A: Water and TFA
B: ACN and TFA

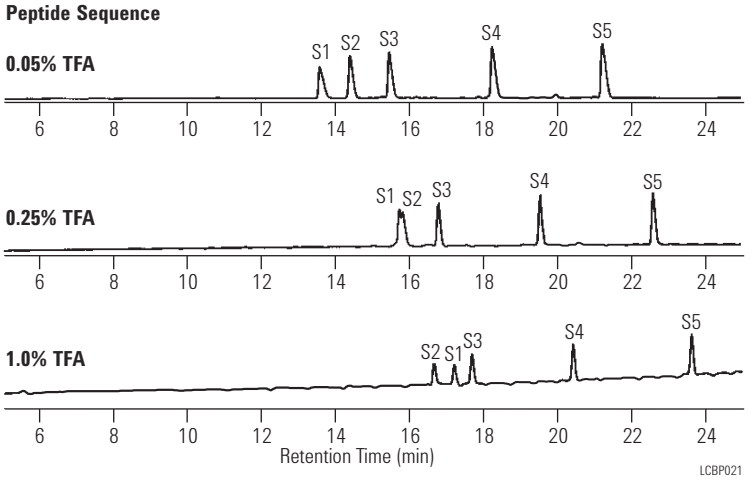
Flow Rate: 1.0 mL/min

Gradient: 0 min 0% B
30 min 30% B

Temperature: 40 °C

Detector: UV 254 nm

Sample: Peptide Standards S1-S5, decapeptides differing slightly in hydrophobicity, 6 µL



Peptides/proteins: Effect of elevated temperature

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

Mobile Phase: A: 5:95
ACN:Water with 0.10% TFA (v/v%)
B: 95:5
ACN:Water with 0.085% TFA (v/v%)

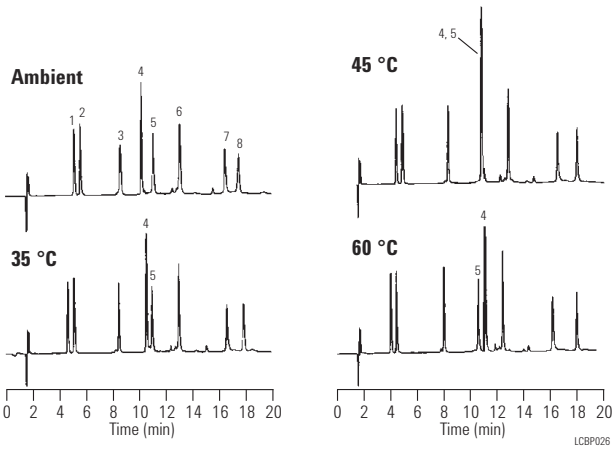
Flow Rate: 1.0 mL/min

Gradient: 15-53% in 20 min, posttime 12 min

Temperature: Ambient – 60 °C

Detector: UV 215 nm

Sample: Polypeptides



1. Leucine Enkephalin
2. Angiotensin II
3. RNase A
4. Insulin (BOV)
5. Cytochrome c
6. Lysozyme
7. Myoglobin
8. Carbonic anhydrase

TIPS & TOOLS

The Agilent 1290 Infinity LC delivers significantly faster results and higher data quality – enabling more informed decisions in shorter time. This higher productivity gives you competitive advantages and provides you a higher return on investment. Calculate for yourself how much you can save by deploying the 1290 Infinity technology. The online method translator and cost savings calculator helps you to transfer your HPLC methods and calculate your cost savings, at **www.agilent.com/chem/hplc2uhplc**

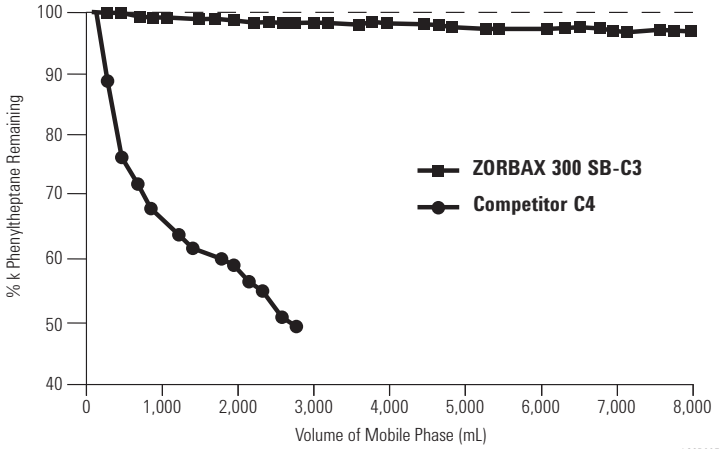
Short-chain ZORBAX 300SB-C3 is stable at low pH, high temperature

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

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 RRHD 300SB-C18**
883995-902
4.6 x 150 mm, 5 µm

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

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

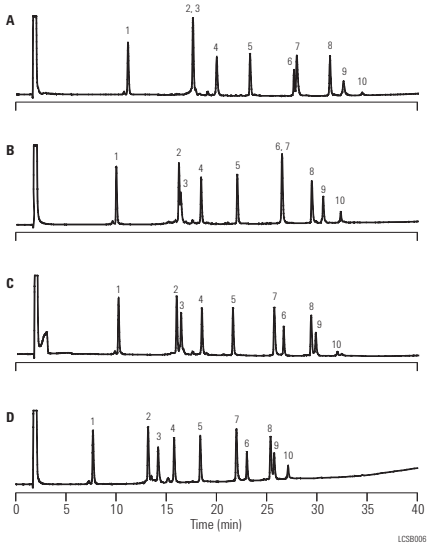
Column D: **ZORBAX 300SB-CN**
883995-905
4.6 x 150 mm, 5 µm

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



1. RNase
2. Insulin
3. Cytochrome c
4. Lysozyme
5. Parvalbumin
6. CDR
7. Myoglobin
8. Carbonic Anhydrase
9. S-100β
10. S-100α

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.

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	300-Diphenyl USP L11
Standard Columns (no special hardware required)								
	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	3.5		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			
	Narrow Bore RRHD	2.1 x 100	1.8	858750-902	858750-906		858750-909	858750-944
	Narrow Bore RRHD	2.1 x 50	1.8	857750-902	857750-906		857750-909	857750-944
	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, 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			840140-901	840140-901	840140-901	840140-901	
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901	

(Continued)

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	300-Diphenyl USP L11
PrepHT Cartridge Columns (require endfittings kit 820400-901)								
PL	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109	
PL	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109	
PL	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909	
PL	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909	
PL	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909	
PL	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901	
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924	
PL	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901	
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			

ZORBAX RRHD 300-Diphenyl

Utilizing the same unique chemistry as the Pursuit 3.5 µm and 5 µm Diphenyl columns, the unique wide pore 300Å Diphenyl phase offers additional selectivity through pi-pi interactions with aromatic amino acids in the primary sequence. Agilent ZORBAX 1.8 µm 300Å Rapid Resolution High Definition (RRHD) columns bring UHPLC performance to the reversed-phase separation of intact proteins and protein digests.

The diphenyl column can be used for:

- Analysis of intact and modified proteins and polypeptides including protein structural analysis
- Detection of post-translational modifications
- Impurity analysis
- Confirming protein identity

The ZORBAX RRHD 300-Diphenyl provides:

- Stability at low pH – allowing you to run your protein and peptide separations down to pH 1 using trifluoroacetic acid (TFA), and formic acid eluents with complete confidence
- Temperature stability – you can run your separations up to 80 °C to improve efficiency and reduce eluent viscosity, without compromising column lifetime
- UHPLC compatible – enabling higher order characterization with reduced analysis time

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp Limits	pH Range	Endcapped	Carbon Load
ZORBAX RRHD 300-Diphenyl	300Å	45 m^2/g	80 °C	1.0-8.0	Yes	1.9%

Specifications represent typical values only

Fast separation of reduced monoclonal antibody

Column: **Agilent ZORBAX RRHD 300-Diphenyl 858750-944 2.1 x 100 mm, 1.8 µm**

Mobile Phase: A: 0.1% TFA in water
B: 80% n-propyl alcohol, 10% ACN, 9.9% water, and 0.1% TFA

Sample: Reduced monoclonal antibody (IgG1) (1.0 mg/mL)

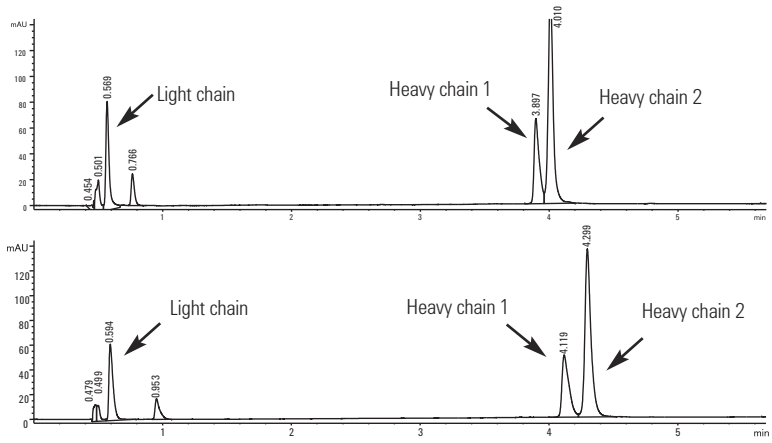
Sample Injection: 2 µL

Flow Rate: 0.5 mL/min

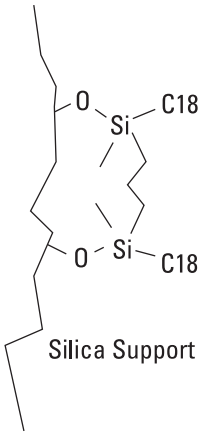
Gradient: 0 min-1%B, 2 min-20% B, 5 min-50% B

Temperature: 74 °C

Detector: UV, 280



Description	Dimensions	Particle Size (µm)	Part No.
ZORBAX RRHD 300-Diphenyl	2.1 x 50	1.8	857750-944
ZORBAX RRHD 300-Diphenyl	2.1 x 100	1.8	858750-944



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

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

Agilent ZORBAX 300Å Extend-C18 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 300Å Extend-C18	300Å	45 m^2/g	60 °C	2.0-11.5	Double	4%

Specifications represent typical values only.

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

TIPS & TOOLS

Selecting the right column is only part of the total solution. Don't forget key supplies such as our wide range of LC lamps. Turn to page 90.

LC/MS analysis of angiotensin on Extend-C18

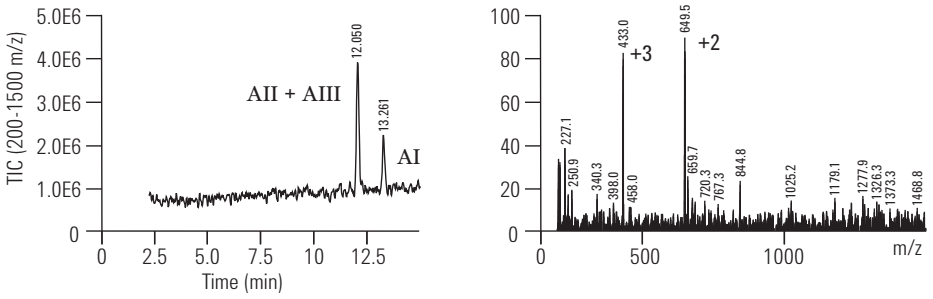
Column: **ZORBAX Extend-C18**
773700-902
2.1 x 150 mm, 5 µm

Mobile Phase: Acidic Conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% acetonitrile (ACN)
Basic Conditions:
A: 10 mM NH_4OH in water
B: 10 mM NH_4OH in 80% ACN

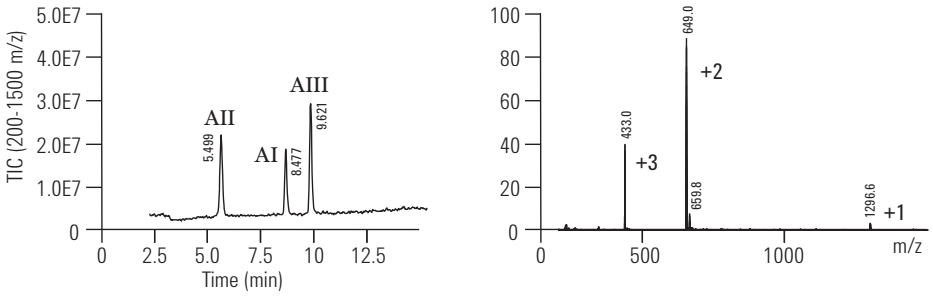
Flow Rate: 0.2 mL/min
Gradient: 15-50% B in 15 min
Temperature: 35 °C

MS Conditions: Pos. Ion ESI- Vf 70 V, Vcap 4.5 kV, N2- 35 psi, 12 L/min., 325 °C
Sample: 2.5 µL sample (50 pmol each) Angiotensin I, II, III

A
Angiotensin I
Max: 10889
Low pH



B
Angiotensin I
Max: 367225
High pH



LC30003

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.

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

Long life at high pH with 300Extend-C18

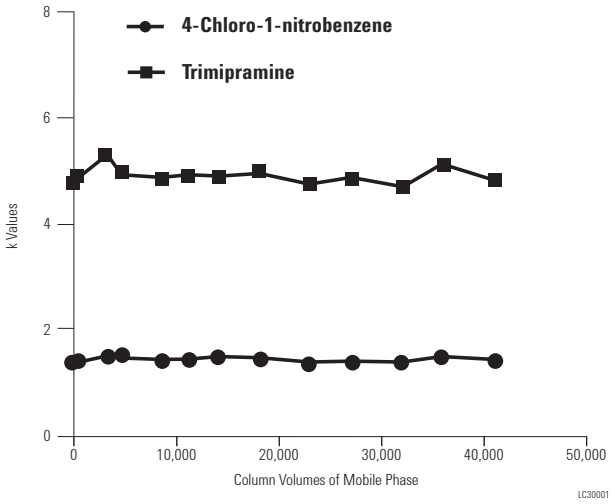
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

Mobile Phase: 20% 20 mM NH_4OH, pH 10.5
80% Methanol

Flow Rate: 1.5 mL/min

Temperature: Aging 24 °C
Tests 40 °C

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



Use ZORBAX Extend-C18 for alternate selectivity at high pH

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

Mobile Phase: A: 0.1% TFA in Water
B: 0.085% TFA in 80% ACN

A: 20 mM NH_4OH in Water
B: 20 mM NH_4OH in 80% ACN

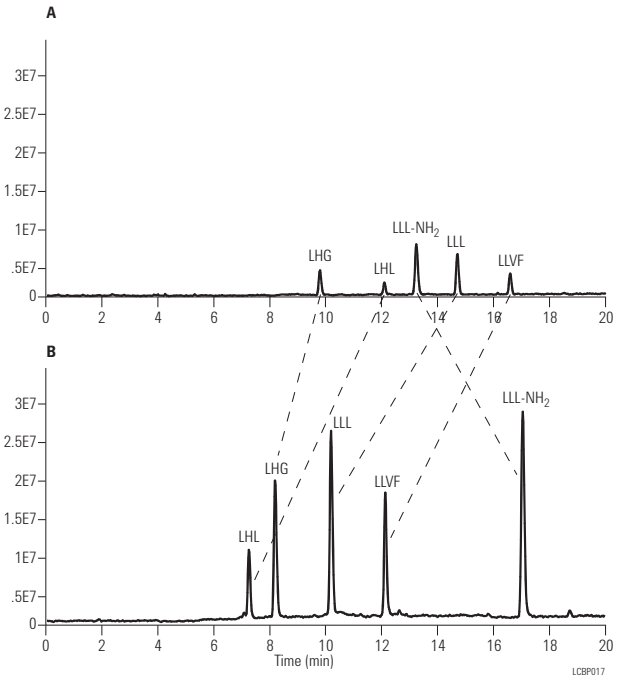
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

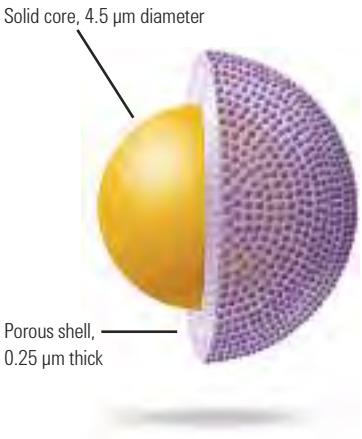
MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV
N_2 – 35 psi, 12 L/min, 300 °C
4 µL (50 ng each peptide)

The Extend column can be used for high pH separations of peptides. At high and low pH, very different selectivity can result. Just by changing pH, a complimentary method can be developed and it is possible to determine if all peaks are resolved. The Extend column can be used at high and low pH, so the complimentary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.



ZORBAX 300Å Extend-C18

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Analytical	4.6 x 250	5	770995-902
	Analytical	4.6 x 150	5	773995-902
	Rapid Resolution	4.6 x 150	3.5	763973-902
	Rapid Resolution	4.6 x 100	3.5	761973-902
	Rapid Resolution	4.6 x 50	3.5	765973-902
	Narrow Bore RR	2.1 x 150	3.5	763750-902
	Narrow Bore RR	2.1 x 100	3.5	761775-902
	Narrow Bore RR	2.1 x 50	3.5	765750-902
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-932
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-932
ZGC	Guard Hardware Kit			820999-901
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



Poroshell 300

- UHPLC separations of biomolecules with superficially porous particles
- 300Å pore provide 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

Agilent Poroshell 300 columns are ideal for fast separations of proteins and peptides because the superficially porous 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. However, Poroshell columns use a superficially porous particle made with a thin layer of porous silica, 0.25 µm thick, 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 possible with 400/600 bar HPLC systems, including the Agilent 1260 Infinity Bio-inert. 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 2-11 for unique separations. These columns can be used for analytical protein separations as well as LC/MS separations.

Poroshell 300 Columns

Column Specifications

Bonded Phase	Pore Size	Temp. Limits*	pH Range	Endcapped
Poroshell 300SB-C18, C8, C3	300Å	90 °C	1.0-8.0	No
Poroshell 300Extend-C18	300Å	40 °C above pH 8 60 °C below pH 8	2.0-11.0	Yes

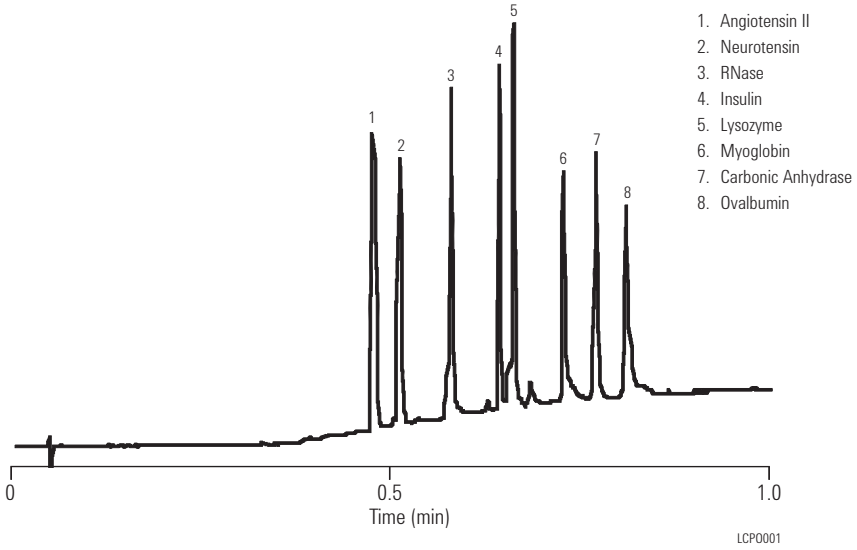
Specifications represent typical values only.

*300StableBond 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.

Poroshell 300 columns separate proteins and peptides in seconds

Column:	**Poroshell 300SB-C18 660750-902 2.1 x 75 mm, 5 µm**
Mobile Phase:	A: 0.1% TFA in H_20 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

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



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

TIPS & TOOLS

Further information can be found in the following publications:

Poroshell 300SB-C18 (publication # 5988-2100ENUS)

Rapid HPLC Analysis of Monoclonal Antibody IgG_1 Heavy Chains Using ZORBAX Poroshell 300SB-C8 (publication # 5989-0070EN)

Use of Temperature to Increase Resolution in the Ultrafast HPLC Separation of Proteins with ZORBAX Poroshell 300SB-C8 HPLC Columns (publication # 5989-0589EN)

Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS (publication # 5989-0683EN)

www.agilent.com/chem/library

Reduce peptide map analysis time by 90% with Poroshell 300SB

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

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

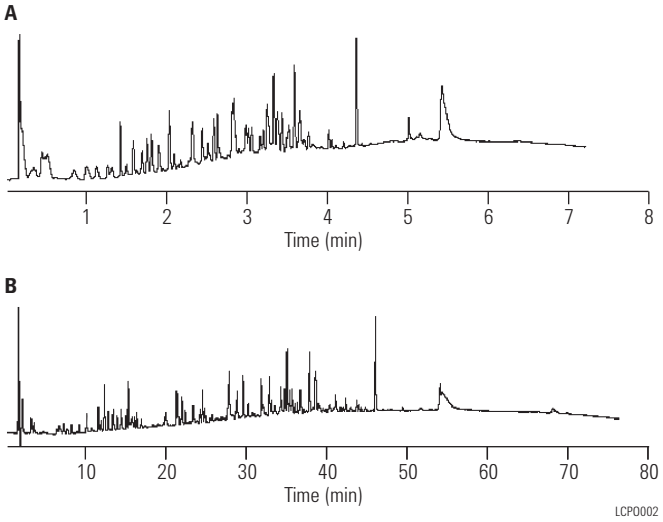
Mobile Phase: A: 95% H_2O, 5% ACN, 0.1% TFA
B: 5% H_2O, 95% ACN, 0.07% TFA

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 one hour or more to complete. With Poroshell columns, the same complex separation can be completed in 1/10th the time.

MicroBore Poroshell 300 columns provide maximum sensitivity for LC/MS

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

Mobile Phase: A: Water + 0.1% Formic Acid
B: ACN + 0.1% Formic Acid

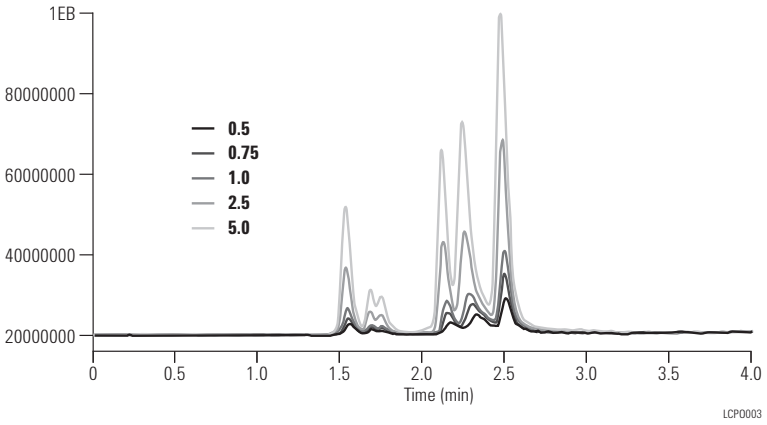
Flow Rate: 600 µL/min

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 L/min
Drying Gas Temperature: 350 °C
Nebulizer: 45 psi
Fragmentor Volatage: 140 V
Scan: 600-2500
Stepsize: 0.15 amu
Peak width: 0.06 min

Sample: 1 µL



With narrow bore diameters of 2.1 mm, 1.0 mm, and 0.5 mm, 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.

Monoclonal IgG1 chains: Separation on Poroshell 300SB-C8

Column: **Poroshell 300SB-C8**
660750-906
2.1 x 75 mm, 5 µm

Mobile Phase: A: 90% water:
10% ACN + 3 mL/L of MW 300 PEG
B: 10% water:
90% ACN + 3 mL/L of MW 300 PEG

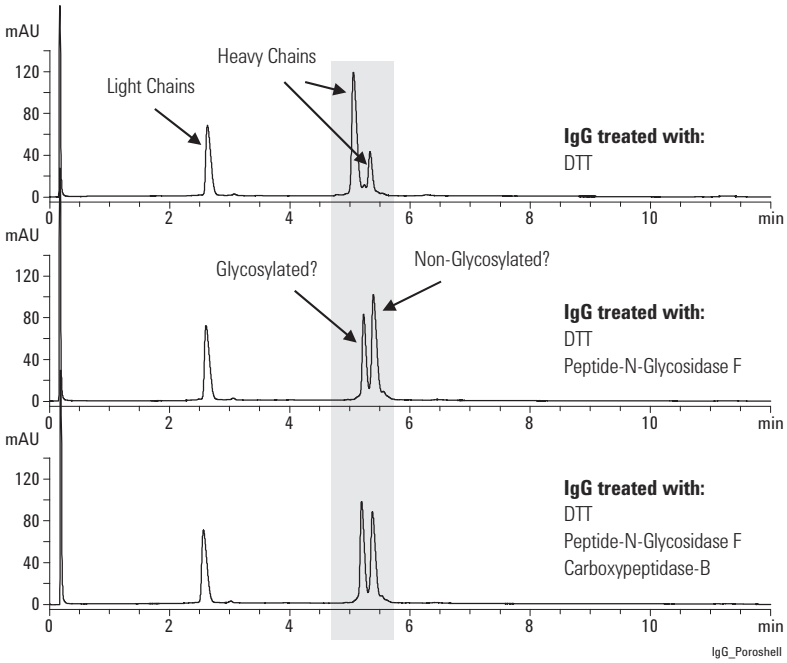
Flow Rate: 1.0 mL/min

Gradient: 0 min 25% B
10 min 40% B
10.1 min 25% B
12 min 25% B

Temperature: 70 °C

Sample: Monoclonal IgG1

Courtesy of:
Novartis Pharma,
Biotechnology, Basel
Dr. Kurt Forrer
Patrik Roethlisberger



TIPS & TOOLS

Agilent offers an extensive selection of certified chromatography sample vials including polypropylene and deactivated and siliconized glass.
For more information see (publication # 5990-9022EN).

www.agilent.com/chem/library

Protein elution pattern on ZORBAX Poroshell 300SB-C8

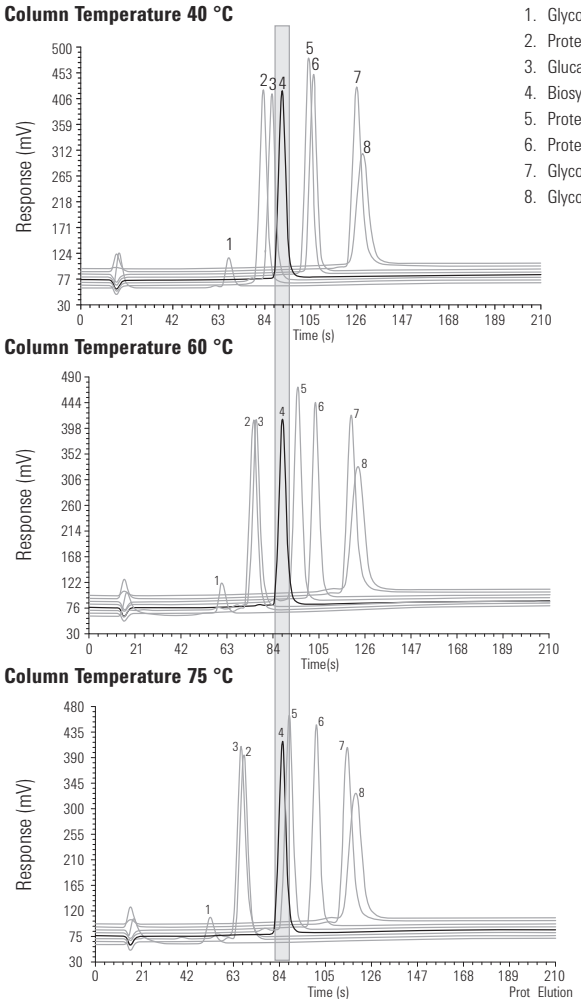
Column: **Poroshell 300SB-C8 660750-906 2.1 x 75 mm, 5 µm**

Mobile Phase: A: 0.1% TFA in H_20
B: 0.1% TFA in ACN

Flow Rate: 1.0 mL/min

Gradient: B: 20 to 70% in 3 min

Detector: UV (214 nm)



1. Glycoprotein X, MW ~ 22 kDa
2. Protein I, MW ~ 4 kDa
3. Glucagon, MW ~ 3.5 kDa
4. Biosynthetic human insulin, MW ~ 6 kDa
5. Protein J, MW ~ 3 kDa
6. Protein K, MW ~ 6 kDa
7. Glycoprotein Y, MW ~ 45 kDa
8. Glycoprotein Z, MW ~ 30 kDa

Poroshell 300

Hardware	Description	Size (mm)	Particle Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
	Narrow Bore	2.1 x 75	5	660750-902	660750-906	660750-909	670750-902
	MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
	Capillary	0.5 x 75	5		5065-4468		
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821075-920	821075-918	821075-924	
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	
	MicroBore Guard, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	5185-5968

Poroshell 120

- 120Å pore size for shorter chain peptide mapping
- UHPLC performance on 600 bar systems
- Up to 90% of the efficiency of sub-2 µm
- 2X the efficiency of 3.5 µm
- Up to 50% less pressure than sub-2 µm columns

Agilent Poroshell 120 columns are a 2.7 µm particle with a 1.7 µm solid core and 0.5 µm porous outer layer. This small particle size provides high efficiency, similar to sub-2 µm columns, but with 40-50% less pressure. These high efficiency, high resolution columns can be used on any type of LC. The porous outer layer and solid core limit diffusion distance and improve separation speed while the narrow particle size distribution improves efficiency and resolution. The columns can support high pressure and multiple columns can be used for the highest resolution and efficiency possible. The smaller 120Å pore size is ideal for fast high resolution analysis of small hydrophilic peptides in protein digests.

Column Specifications

Bonded Phase	Pore Size	Temp Limits	pH Range	Endcapped	Carbon Load
EC-C18	120Å	60 °C	2.0-8.0	Double	10%
SB-C18	120Å	90 °C	1.0-8.0	No	8%

Specifications represent typical values only

For information on the full family of Poroshell 120 phases, see page 228.

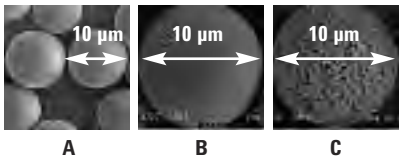
Poroshell 120

Description	Size (mm)	Particle Size (µm)	EC-C18 USP L1	SB-C18 USP L1
Analytical	4.6 x 150	2.7	693975-902	683975-902
Analytical	4.6 x 100	2.7	695975-902	685975-902
Solvent Saver	3.0 x 150	2.7	693975-302	683975-302
Solvent Saver	3.0 x 100	2.7	695975-302	685975-302
Narrow Bore	2.1 x 150	2.7	693775-902	683775-902
Narrow Bore	2.1 x 100	2.7	695775-902	685775-902

PLRP-S

- Contain durable and resilient polymer particles that deliver reproducible results over longer lifetimes
- Thermally and chemically stable
- Comply with USP L21 designation
- Used in bioscience, chemical, clinical research, energy, environmental, food and agriculture, material science and pharmaceutical industries
- Pore sizes (100Å-4000Å) for separations of small molecules to large complexes and polynucleotides

The PLRP-S family of columns consists of a range of pore sizes and particle sizes, all with identical chemistry and fundamental adsorptive characteristics. The particles are inherently hydrophobic, therefore no bonded phase, alkyl ligand is required for reversed-phase separations. This gives a highly reproducible material that is free from silanols and heavy metal ions. Columns within the extensive product range are suitable for nano/capillary separations, including both bottom-up and top-down proteomics, analytical separations, and preparative purifications. In addition, process columns can be packed with bulk media.



Scanning electron micrographs (SEM) of PLRP-S 10 µm particles.
The difference in pore size is clearly seen.
A is the small pore 100Å
B the larger pore 300Å
C the gigaporous 4000Å

Column Specifications	
pH Range	1-14
Buffer Content	Unlimited
Organic Modifier	1-100%
Temperature Limits	200 °C
Maximum Pressure	5-8 µm: 3000 psi (210 bar)
	3 µm: 4000 psi (300 bar)

PLRP-S Applications	
Pore Size	**Application**
100Å	Small molecules/peptides/oligonucleotides
300Å	Recombinant peptides/proteins
1000Å	Large proteins
4000Å	DNA/high speed

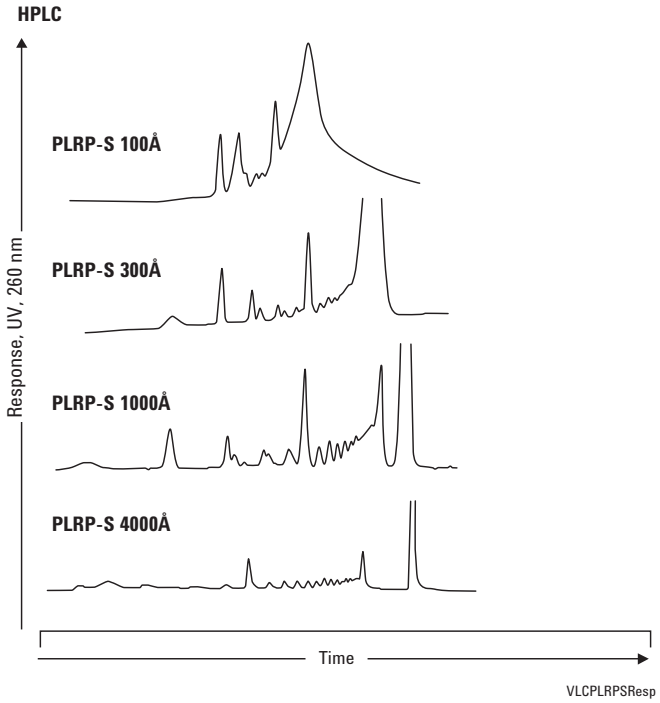
HPLC of 25 bp DNA ladder

Column: **PLRP-S, 2.1 x 150 mm**

Mobile Phase: A: 0.1 M TEAA
B: 0.1 M TEAA in 50% water:50% ACN

Flow Rate: 200 µL/min

Gradient: 12.5-50% B in 150 min



Polyethylene glycols

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: A: Water
B: ACN

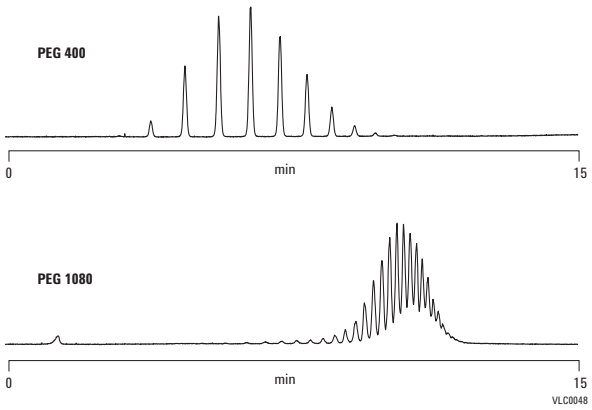
Gradient: 10-30% B in 12 min, held at 30% B for 3 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

Detector: ELS (neb=50 °C, evap=70 °C, gas=1.6 SLM)



Exploiting chemical stability – TFA concentration

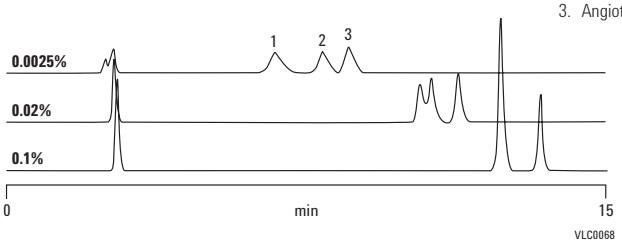
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: A: TFA (various %) in water
B: TFA (various %) in ACN

Gradient: Linear 12-40% B in 15 min

Flow Rate: 1.0 mL/min

Detector: ELS (neb=75 °C, evap=85 °C, gas=1.0 SLM)



1. Angiotensin III
2. Angiotensin II
3. Angiotensin I

Selectivity in peptide RP-LC

Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

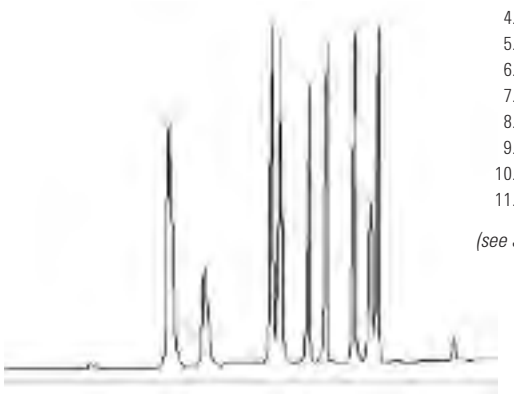
Mobile Phase: A: 0.1% TFA/1% 2-Propanol/Water
B: 0.1% TFA/1% 2-Propanol/ACN

Flow Rate: 1.0 mL/min

Gradient: 95% A (0-3 min) to 50% A (13 min)

Detector: UV, 220 nm

Good separation of peptide standards on Agilent PLRP-S



1. YG
2. GYG
3. PY
4. YV
5. YY
6. GLY
7. YF
8. GFL
9. YGGFM
10. Oxalic acid (marker)
11. Benzoic acid (marker)

(see J.Chromatography 512 (1990) 315-23)

Exploiting chemical stability – NH_4OH concentration

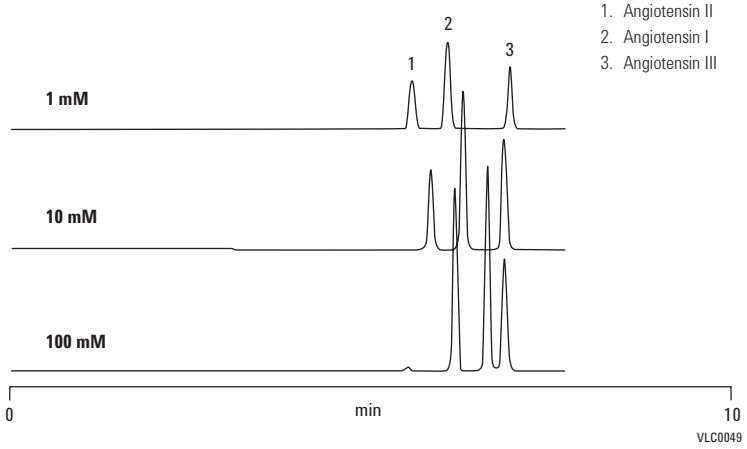
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: A: NH_4OH (various mM) in water
B: NH_4OH (various mM) in ACN

Gradient: Linear 10-100% B in 15 min

Flow Rate: 1.0 mL/min

Detector: ELS (neb=80 °C, evap=85 °C, gas=1.0 SLM)



Alberta Peptide Institute test mix

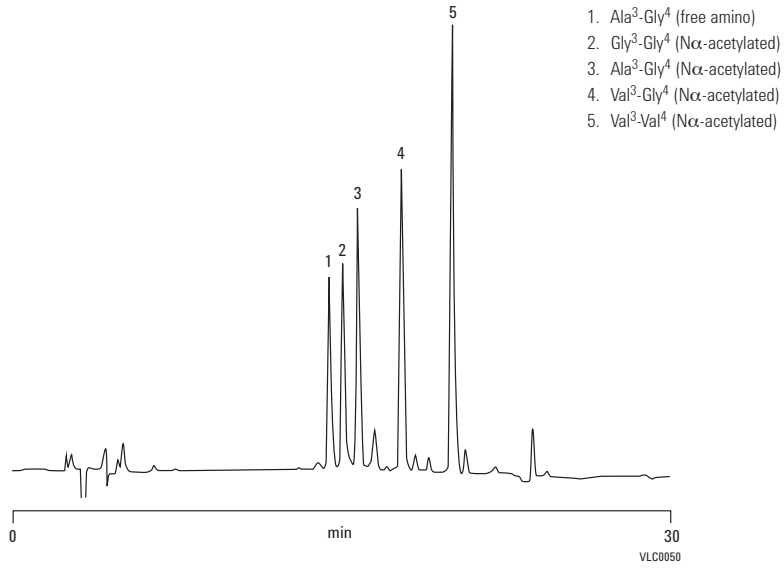
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 70% water:30% ACN

Gradient: 0-100% B in 30 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



Whey proteins in dairy samples – milk

Column: **PLRP-S 300Å**
PL1512-3801
4.6 x 150 mm, 8 µm

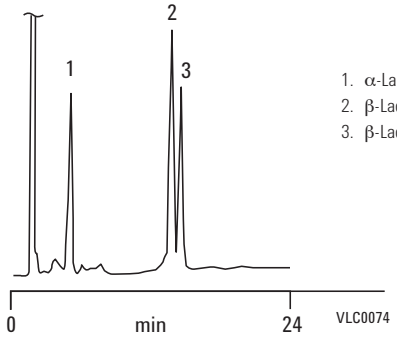
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

Gradient: 36-48% B, 0-24 min, 48-100% B, 24-30 min
100% B, 30-35 min, 100-36% B, 35-40 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Detector: UV, 220 nm



1. α-Lactalbumin
2. β-Lactoglobulin (B chain)
3. β-Lactoglobulin (A chain)

Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion-pair reversed-phase HPLC

Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

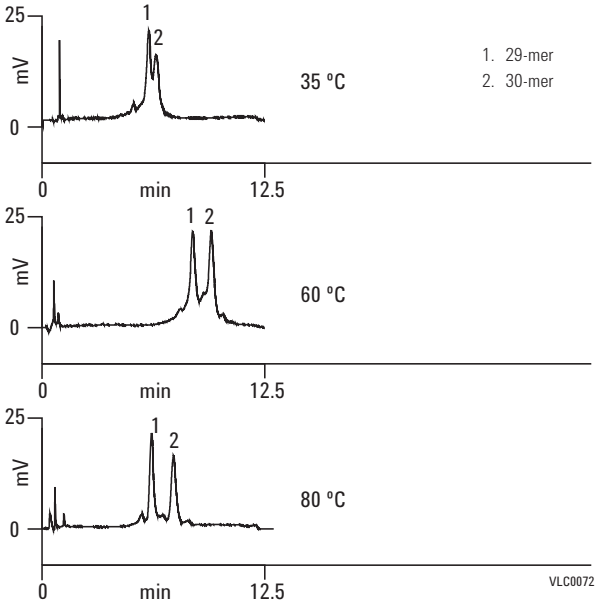
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

Gradient: 5% change in buffer B over 5 min

Flow Rate: 1.0 mL/min

Temperature: 35 °C, 60 °C, or 80 °C

Detector: UV, 254 nm



1. 29-mer
2. 30-mer

Large fibrous proteins

Column: **PLRP-S 300Å**
PL1512-3801
4.6 x 150 mm, 8 µm

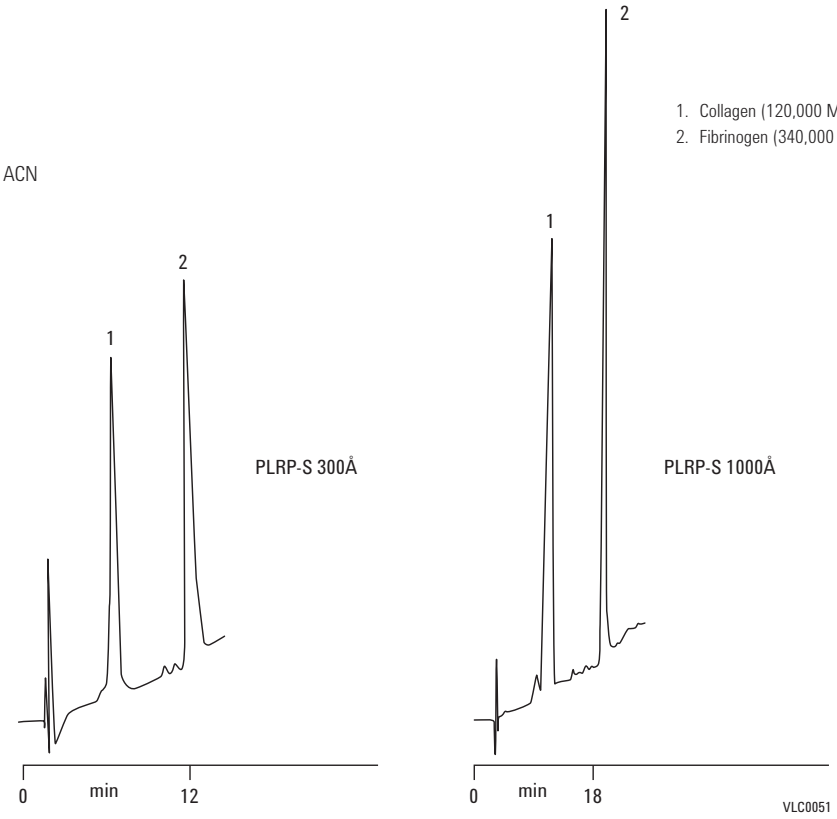
Column: **PLRP-S 1000Å**
PL1512-3802
4.6 x 150 mm, 8 µm

Mobile Phase: A: 0.25% TFA in water
B: 0.25% TFA in 5% water:95% ACN

Flow Rate: 1.0 mL/min

Gradient: 20-60% B in 15 min

Detector: UV, 220 nm



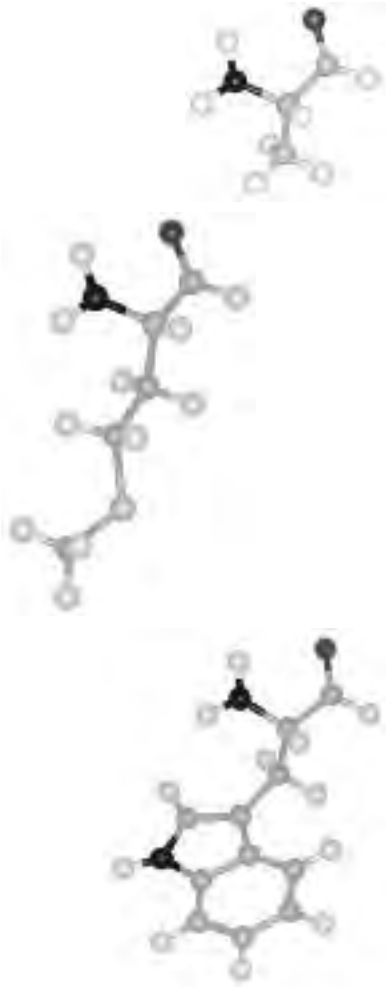
PLRP-S HPLC Columns

Hardware	Size (mm)	Particle Size (µm)	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
	4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
	4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
	4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
	4.6 x 250	5	PL1512-5500	PL1512-5501		
	4.6 x 150	5	PL1111-3500	PL1512-3501		
	4.6 x 50	5	PL1512-1500	PL1512-1501	PL1512-1502	PL1512-1503
	4.6 x 150	3	PL1512-3300	PL1512-3301		
	4.6 x 50	3	PL1512-1300	PL1512-1301		
	2.1 x 250	8		PL1912-5801		
	2.1 x 150	8		PL1912-3801	PL1912-3802	PL1912-3803
	2.1 x 50	8		PL1912-1801	PL1912-1802	PL1912-1803
	2.1 x 250	5	PL1912-5500	PL1912-5501		
	2.1 x 150	5	PL1912-3500	PL1912-3501		
	2.1 x 50	5	PL1912-1500	PL1912-1501	PL1912-1502	PL1912-1503
	2.1 x 150	3	PL1912-3300	PL1912-3301		
	2.1 x 50	3	PL1912-1300	PL1912-1301		
	1.0 x 50	8			PL1312-1802	
	1.0 x 50	5	PL1312-1500		PL1312-1502	
	1.0 x 10	5			PL1C12-2502	
	1.0 x 150	3	PL1312-3300			
	1.0 x 50	3	PL1312-1300			
PL	PLRP-S Guard Cartridges for 5 x 3 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
PL	Guard Cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

TIPS & TOOLS

For prep columns and media ordering information, turn to pages 470-471.

For microbore columns ordering information, turn to page 463.



Amino Acid Analysis (AAA) Columns and Supplies

ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

- High resolution and rapid analysis of 24 amino acids
- 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 Agilent 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 8 min (7 min analysis time) on a 50 mm 1.8 µm column, 14 min (9 min analysis time) on shorter, 75 mm length columns and 24 min (18 min analysis time) on the 150 mm 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.

ZORBAX Eclipse Plus C18 columns are another excellent choice for Amino Acid Analysis. For more information about ZORBAX Eclipse Plus Columns, see page 248.

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			820999-901

TIPS & TOOLS

Further information can be found in the following publication:

High-Speed Amino Acid Analysis (AAA) on 1.8 µm Reversed-Phase (RP) Columns (publication # 5989-6297EN)

www.agilent.com/chem/library

Amino Acid Standards

Each amino acid standard contains the following amino acids:

- Glycine
- L-cysteine
- 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, P/N 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 (Dithiodiproprionic) reagent, for analysis of cysteine, 5 g	5062-2479

High resolution of 24 amino acids using ZORBAX Eclipse-AAA protocol

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

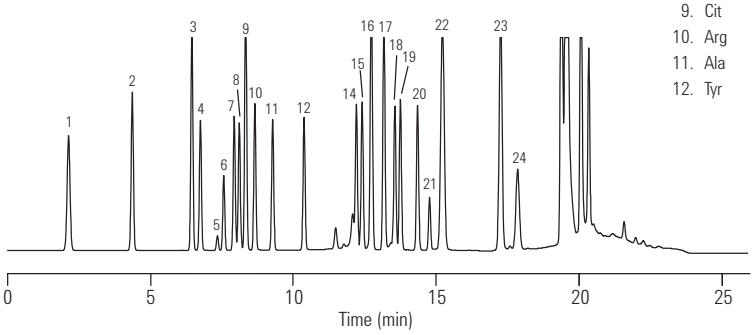
Mobile Phase: A: 40 mM Na_2HPO_4, pH 7.8
B: ACN:MeOH:Water, 45:45:10 v/v

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: Fluorescence

Sample: 24 Amino Acids



LCPAH01

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

Ion-Exchange Chromatography

Purify proteins and other charged molecules

Ion-exchange chromatography (IEX) is a highly sensitive technique that allows you to separate ions and polar molecules based on their charge. Like SEC, IEX can be used to separate proteins in their native state.

Applying IEX to charge variant analysis

During production and purification, antibodies can exhibit changes in charge heterogeneity as a result of amino acid substitutions, glycosylation, phosphorylation, and other post-translational or chemical modifications. Because these changes can impact stability and activity – or cause immunologically adverse reactions – the analysis of charge heterogeneity in monoclonal antibody (MAb) preparations is critical to biopharmaceuticals.

In protein analysis, charge variations at a given pH indicate a change in the primary molecular structure – resulting in additional forms of the protein in question. These are called isoforms (or charge variants), and can be resolved by IEX chromatography. IEX is also useful as a preparative technique.

The pages that follow describe Agilent's family of weak and strong ion-exchangers – both anionic and cationic.

- **Agilent non-porous Bio IEX columns** are designed for high-resolution, high-efficiency, and high-recovery separations.
- **Agilent Bio MAb columns** are optimized for separating charge isoforms of monoclonal antibodies.
- **Agilent porous IEX columns (PL-SAX and PL-SCX)** are chemically stable, and are available in two pore sizes – allowing you to separate peptides, oligonucleotides, and very large proteins.
- **Bio-Monolith IEX columns** are uniquely suited to separating antibodies, viruses, and DNA.

Ion-Exchange Column Selection

Application	Agilent Columns	Notes
Monoclonal antibodies	Agilent Bio MAb	Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution charge-based separations of monoclonal antibodies.
Peptides and proteins	Agilent Bio IEX	Agilent Bio Ion-Exchange columns are packed with polymeric, nonporous, ion-exchange particles. Bio IEX columns are designed for high resolution, high recovery and highly efficient separations.
Proteins, peptides and deprotected synthetic oligonucleotides	PL-SAX • 1000Å • 4000Å	The strong anion-exchange functionality, covalently linked to a fully porous chemically stable polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. The 5 µm media delivers separations at high resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins and peptides	PL-SAX 1000Å	
Very large biomolecules/high speed	PL-SAX 4000Å	
Small peptides to large proteins	PL-SCX • 1000Å • 4000Å	PL-SCX is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation and purification of a wide range of biomolecules. The 5 µm media delivers separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins	PL-SCX 1000Å	
Very large biomolecules/high speed	PL-SCX 4000Å	
Antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro biomolecules	Bio-Monolith • Bio-Monolith QA • Bio-Monolith DEAE • Bio-Monolith SO_3 • Bio-Monolith Protein A	Strong cation-exchange, strong and weak anion-exchange, and Protein A phases. Bio-Monolith HPLC columns are compatible with preparative LC systems, including Agilent 1100 and 1200 HPLC systems.
Viruses, DNA, large proteins	Bio-Monolith QA	
Plasmid DNS, bacteriophages	Bio-Monolith DEAE	
Proteins, antibodies	Bio-Monolith SO_3	

Agilent Bio MAb HPLC Columns

- A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) non-porous bead
- Particles grafted with a hydrophilic, polymeric layer, virtually eliminating non-specific binding of antibody proteins
- A different process is used to layer the weak cation-exchange phase to the particle making it a higher density than the Agilent Bio WCX column particles
- Specifically designed for the separation of charge isoforms of monoclonal antibodies

Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution, charge-based separations of monoclonal antibodies. Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: phosphate, tris, MES and acetate.

Bio MAb columns are available in 1.7, 3, 5 and 10 µm sizes, providing higher resolution with smaller particles.

Column Specifications

Bonded Phase	ID	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
Weak Cation-Exchange (carboxylate)	2.1 and 4.6 mm	1.7, 3, 5 and 10 µm	2-12	80 °C	0.1-1.0 mL/min

TIPS & TOOLS

Capillary electrophoresis is an alternative technique to liquid chromatography for the separation of charged isoforms. Further information can be found in the following Technical Note:

Capillary electrophoreseis focusing on the Agilent Capillary Electrophoresis system (publication # 5989-9852EN)

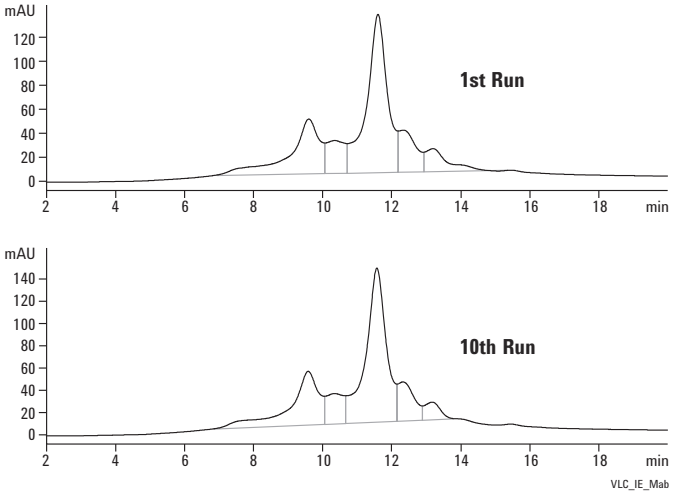
www.agilent.com/chem/library

Consistent ion-exchange MAb separation

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

Buffer:	A: Sodium phosphate buffer, 20 mM B: Buffer A + 400 mM NaCl
Gradient:	15-35% Buffer B from 0-30 min
Flow Rate:	0.65 mL/min
Sample:	CHO-humanized MAb, 1 mg/mL
Injection:	2.5 µL
Detector:	UV 220 nm
Temperature:	Ambient

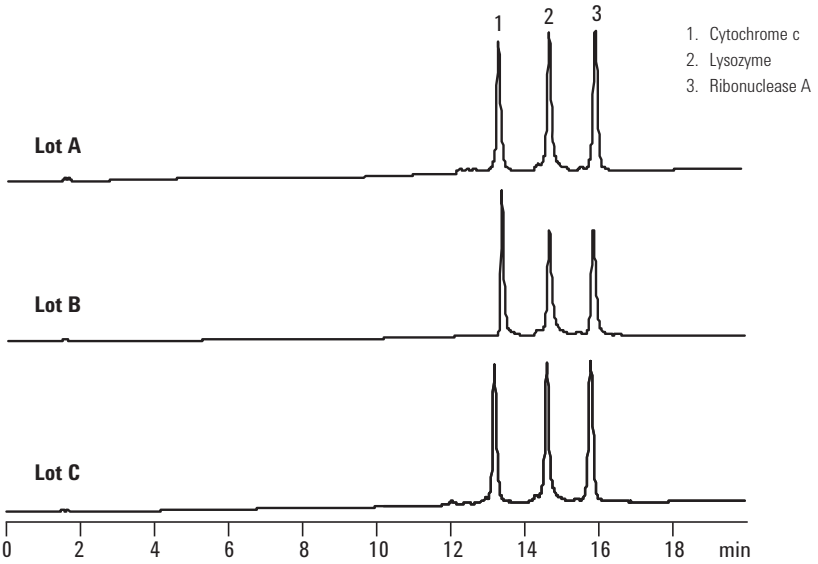
To provide a metal free flow path, Bio MAb PEEK columns are available.



Virtually eliminate retention time variations

Column: **Bio MAb, stainless steel**
5190-2413
4.6 x 250 mm, 10 µm

Mobile Phase:	A: 10 mM phosphate, pH 6.0 B: A + 1.0 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	0-100% B in 42 min
Temperature:	25 °C
Detector:	UV 214 nm



The combination of well-controlled resin production, column surface chemistry, and column packing virtually eliminates retention time variations from column-to-column and lot-to-lot.

Charge isoform analysis of monoclonal antibodies

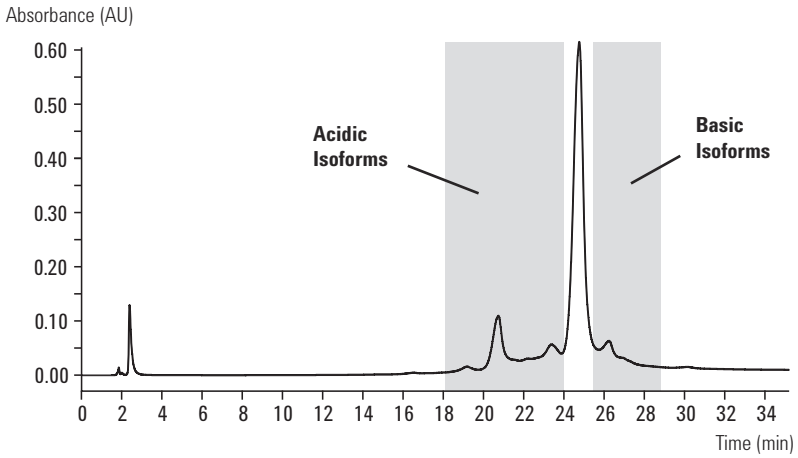
Column: **Bio MAb, PEEK 5190-2407 4.6 x 250 mm, 5 µm**

Mobile Phase: A: 10 mM Sodium Phosphate, pH 7.50
B: A + 100 mM NaCl, pH 7.50

Flow Rate: 0.8 mL/min

Gradient: 15-95% B in 60 min

Sample: 5 µL, 5 mg/mL, MAb



High resolution separation of acidic and basic charge variants using the Agilent Bio MAb NP5 column

Agilent Bio MAb HPLC Columns

Size (mm)	Particle Size (µm)	Bio MAb PEEK	Pressure Limit	Bio MAb Stainless Steel	Pressure Limit
4.6 x 250	10	5190-2415	275 bar, 4000 psi	5190-2413	275 bar, 4000 psi
4.6 x 50, Guard	10	5190-2416	275 bar, 4000 psi		
4.6 x 250	5	5190-2407	400 bar, 5800 psi	5190-2405	413 bar, 6000 psi
4.6 x 50, Guard	5	5190-2408	400 bar, 5800 psi		
4.6 x 50	3			5190-2403	551 bar, 8000 psi
4.6 x 50	1.7			5190-2401	600 bar, 8700 psi
4.0 x 10, Guard	10			5190-2414	275 bar, 4000 psi
4.0 x 10, Guard	5			5190-2406	413 bar, 6000 psi
4.0 x 10, Guard	3			5190-2404	551 bar, 8000 psi
4.0 x 10, Guard	1.7			5190-2402	600 bar, 8700 psi
2.1 x 250	10	5190-2419	275 bar, 4000 psi		
2.1 x 50, Guard	10	5190-2420	275 bar, 4000 psi		
2.1 x 250	5	5190-2411	400 bar, 5800 psi		
2.1 x 50, Guard	5	5190-2412	400 bar, 5800 psi		

Agilent Bio IEX HPLC Columns

- Highly cross-linked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, eliminating nonspecific binding
- Uniform, densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer (multiple ion-exchange groups per anchoring) to increase column capacity
- Particles, coating and bonding are resistant to high pressures, promoting higher resolution and faster separations
- Multiple ion-exchange groups are captured on one anchoring to increase capacity

Agilent Bio IEX HPLC columns are packed with polymeric, nonporous, ion-exchange particles and are designed for high resolution, high recovery and highly efficient separations of peptides, oligonucleotides and proteins.

The Bio IEX family offers strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX) and weak anion-exchange (WAX) phases. All phases are available in 1.7, 3, 5 and 10 µm non-porous particles sizes.

Column Specifications

Bonded Phase	ID	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
SCX (Strong cation-exchange) - SO_3H	2.1 and 4.6 mm	1.7, 3, 5 and 10 µm	2-12	80 °C	0.1-1.0 mL/min
WCX (Weak cation-exchange) - COOH					
SAX (Strong anion-exchange) - $N(CH_3)_3$					
WAX (Weak cation-exchange) - $N(C_2H_5)_2$					

TIPS & TOOLS

More information is a click away. We have a variety of educational primers, application notes, maintenance guides, and literature available from Agilent for free.

To learn more, visit **www.agilent.com/chem/library**

Exceptional separating power

Column: **Agilent Bio SCX, stainless steel**
5190-2423
4.6 x 50 mm, 3 µm

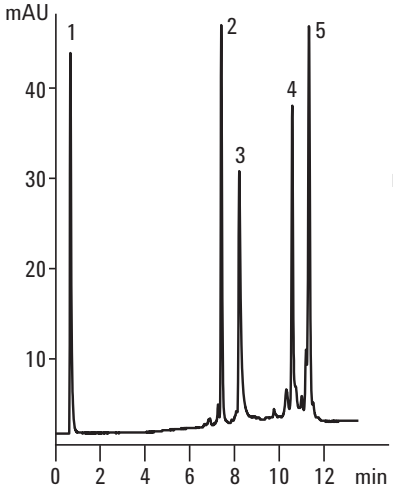
Buffer: 10 mM Phosphate, pH 6.0

Flow Rate: 0.5 mL/min

Gradient: 0-1.0 M NaCl, 15 min

Detector: 280 nm

The hydrophilic, polymeric layer and densely packed ion-exchange functional groups provide extremely sharp peak shapes and high resolution of a mixture of proteins with a broad range of isoelectric points (pI).



1. Ovalbumin, 4.6 pI
2. Ribonuclease A, 8.7 pI
3. Cytochrome c, 9.6 pI
4. Aprotinin, 10.0 pI
5. Lysozyme, 11.0 pI

N > 100,000/50 mm for Lysozyme

Separation of protein standards on Agilent 3 µm ion-exchange columns by cation-exchange chromatography

Column A: **Agilent Bio SCX, NP 3, 4.6 x 50 mm, SS**

Column B: **Agilent Bio WCX, NP 3, 4.6 x 50 mm, SS**

Column C: **Agilent Bio MAb, NP 3, 4.6 x 50 mm, SS**

Mobile Phase: A: 10 mM $NaH_2PO4.2H_2O$, pH 5.70
B: A + 1 M NaCl

Flow Rate: 0.5 mL/min

Gradient: 0 min - 100% A : 0% B
25 min - 0% A : 100% B

Temperature: Ambient

Detector: Agilent 1260 Infinity Bio-inert Quaternary LC with diode array detector at 220 nm

Sample: Cytochrome c, ribonuclease A, lysozyme and protein mix

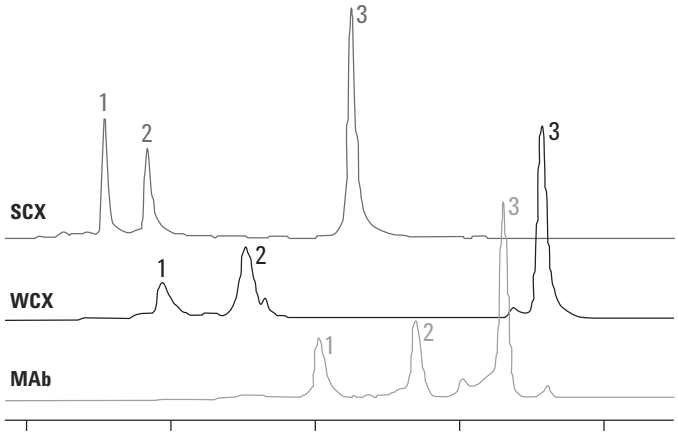


Illustration that Bio WCX, SCX and MAb columns are capable of producing protein separations

Agilent column	Peak number	Peak name	RT [min]	Height [mAU]	Area [mAU*s]	Plates	Width [min]	Resolution
Bio WCX NP, 3 µm	1	Cytochrome c	7.86	124	1833	7844	0.2089	-
	2	RNase A	9.03	241	3358	10800	0.2044	3.32
	3	Lysozyme	13.13	636	7274	44488	0.1466	13.73
Bio SCX NP, 3 µm	1	RNase A	7.06	396	2616	39847	0.0832	-
	2	Cytochrome c	7.66	297	2778	28920	0.1060	1.08
	3	Lysozyme	10.49	763	7186	44828	0.1167	1.37
Bio MAb NP, 3 µm	1	Cytochrome c	10.04	203	2369	21814	0.1600	-
	2	RNase A	11.37	256	2690	33314	0.1467	3.11
	3	Lysozyme	12.59	652	6616	56734	0.1244	5.28

Weak cation-exchange chromatography for P128 therapeutic protein sample on the Agilent 1260 Bio-inert Quaternary LC system using different cation-exchange columns

Column A: **Bio MAb, PEEK**
5190-2407
4.6 x 250 mm, 5 µm

Column B: **Bio MAb, PEEK**
5190-2415
4.6 x 250 mm, 10 µm

Column C: **Brand B WCX-10**
4.0 x 250 mm, 10 µm

Mobile Phase: A: 20 mM sodium phosphate (pH = 6.0)
B:20 mM sodium phosphate (pH = 6.0) containing 1.0 M sodium chloride

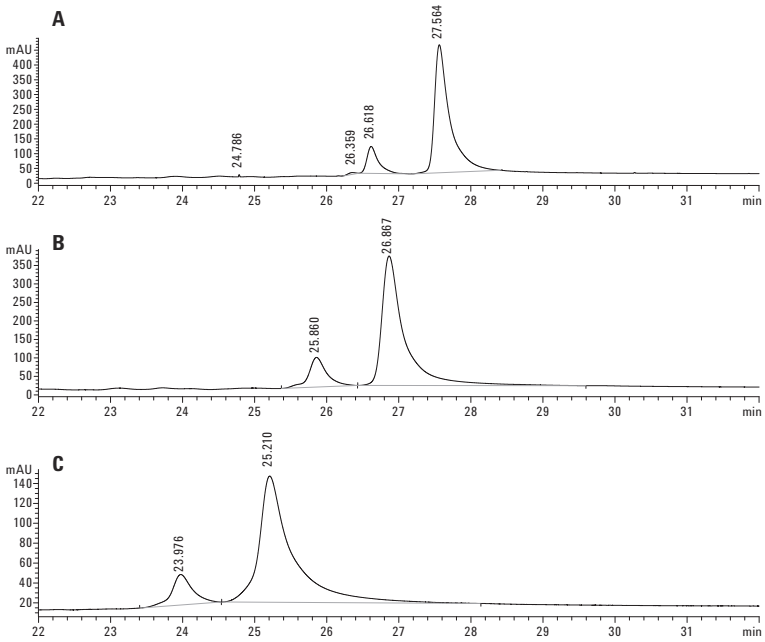
Flow Rate: 0.5 mL/min

Gradient: 10% B 0 min, 35% B 35 min, 10% B 36 min, 10% B 45 min

Detector: UV, 220 nm/4 nm, Reference: Off (data also acquired at 220, 230, 240, and 280 nm)

Sample: P128

Sample was desalted by ultrafiltration and extracted into 20 mM sodium phosphate.



Agilent Bio IEX HPLC Columns, PEEK

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
4.6 x 250	10	275 bar, 4000 psi	5190-2435	5190-2455	5190-2475	5190-2495
4.6 x 50, Guard	10	275 bar, 4000 psi	5190-2436	5190-2456	5190-2476	5190-2496
4.6 x 250	5	400 bar, 5800 psi	5190-2427	5190-2447	5190-2467	5190-2487
4.6 x 50, Guard	5	400 bar, 5800 psi	5190-2428	5190-2448	5190-2468	5190-2488
2.1 x 250	10	275 bar, 4000 psi	5190-2439	5190-2459	5190-2479	5190-2499
2.1 x 50, Guard	10	275 bar, 4000 psi	5190-2440	5190-2460	5190-2480	5190-2500
2.1 x 250	5	400 bar, 5800 psi	5190-2431	5190-2451	5190-2471	5190-2491
2.1 x 50, Guard	5	400 bar, 5800 psi	5190-2432	5190-2452	5190-2472	5190-2492

Agilent Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
4.6 x 250	10	275 bar, 4000 psi	5190-2433	5190-2453	5190-2473	5190-2493
4.6 x 250	5	413 bar, 6000 psi	5190-2425	5190-2445	5190-2465	5190-2485
4.6 x 50	3	551 bar, 8000 psi	5190-2423	5190-2443	5190-2463	5190-2483
4.6 x 50	1.7	600 bar, 8700 psi	5190-2421	5190-2441	5190-2461	5190-2481
4.0 x 10, Guard	10	275 bar, 4000 psi	5190-2434	5190-2454	5190-2474	5190-2494
4.0 x 10, Guard	5	413 bar, 6000 psi	5190-2426	5190-2446	5190-2466	5190-2486
4.0 x 10, Guard	3	551 bar, 8000 psi	5190-2424	5190-2444	5190-2464	5190-2484
4.0 x 10, Guard	1.7	275 bar, 4000 psi	5190-2422	5190-2442	5190-2462	5190-2482

PL-SAX Strong Anion-Exchange Columns

- Small particles deliver excellent chromatographic performance
- Wide range of particle sizes and 2 pore sizes for flexible analysis to scale-up purification
- Exceptional stability for long column lifetime

PL-SAX -$N(CH_3)_3^+$ is ideal for the anion-exchange HPLC separations of proteins, peptides and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable fully porous polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. PL-SAX delivers improved chromatography for self-complementary or G-rich sequences that may associate to form aggregates or hairpin structures. The 5 µm material provides high efficiency separations of n and n-1 sequences. A wide range of particle sizes and column geometries permits analysis scale-up to purification. The strong anion-exchange functionality provides a material with exceptional chemical and thermal stability, even with sodium hydroxide eluents, leading to long column lifetime.

Column Specifications

Bonded Phase	ID (mm)	Particle Size (µm)	Pore Size	pH Stability	Operating Temperature Limit
Strong Anion-Exchange	2.1, 4.6, 7.5, 25, 50 and 100	5, 8, 10 and 30	1000Å and 4000Å	1-14	80 °C

Standard ion-exchange protein separation

Column: **PL-SAX 1000Å**
PL1551-1502
4.6 x 50 mm, 5 µm

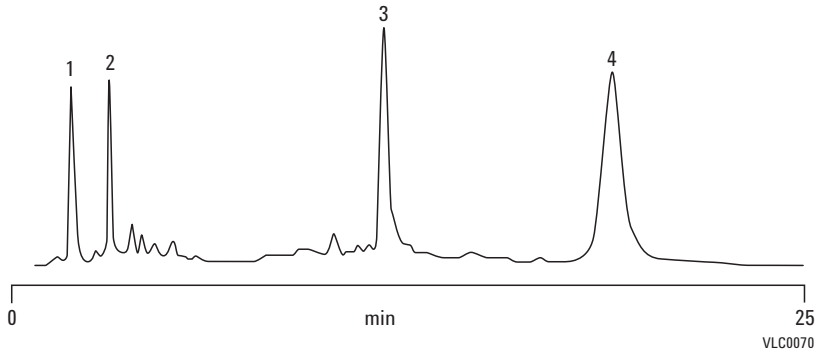
Mobile Phase: A: 10 mM Tris HCl pH 8
B: A+0.35 M NaCl pH 8

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

1. Myoglobin
2. Bovine carbonic anhydrase
3. Ovalbumin
4. Soybean trypsin inhibitor



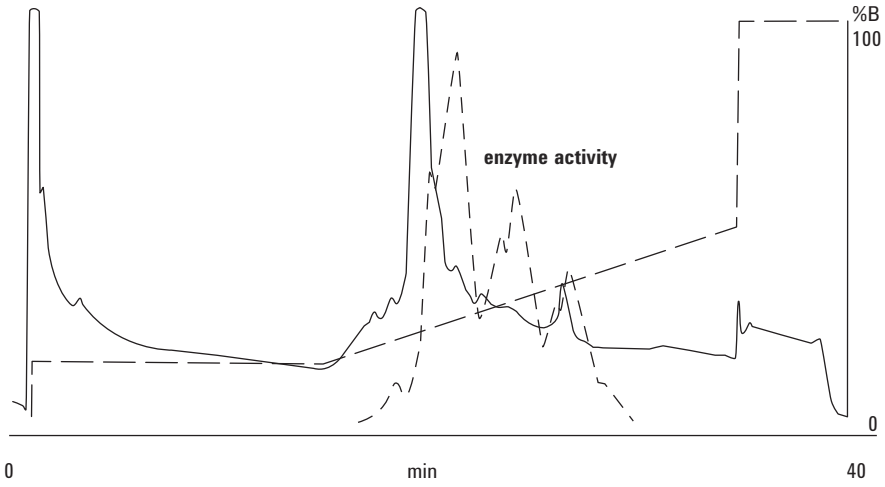
Analysis of choline kinase on PL-SAX 4000Å

Column: **PL-SAX**
PL1551-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 20 mM Tris 5% ethylene glycol, pH 7.5
(The following are required to retain enzyme activity)
1.0 mM Ethylene glycol tetraacetic acid
2.0 mM ß-Mercaptoethanol
0.2 mM Phenylmethylsulfonyl fluoride
B: A + 1 M KCl

Flow Rate: 3.0 mL/min

Detector: UV, 280 nm



Separation courtesy of T Porter, Purdue University, USA

Analysis of representative whey proteins

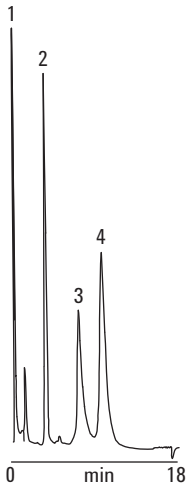
Column: **PL-SAX 1000Å**
PL1551-1802
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.02 M Tris HCl, pH 7
B: A + 0.5 M CH_3COONa, pH 7

Flow Rate: 1.0 mL/min

Gradient: Linear 0-50% B in 10 min

Detector: UV, 280 nm



1. Carbonic anhydrase
2. α-lactalbumin
3. β-lactoglobulin B
4. β-lactoglobulin A

High resolution separation of a Poly-T-Oligonucleotide size standard spiked with 10-mer, 15-mer, 30-mer and 50-mer (main peaks)

Column: **PL-SAX 1000Å**
PL1551-1802
4.6 x 50 mm, 8 µm

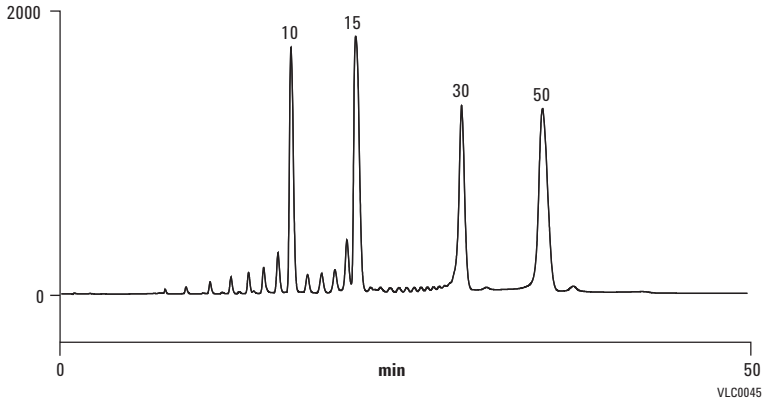
Mobile Phase: A: 7:93 v/v ACN: 0.1 M TEAA, pH 8.5
B: 7:93 v/v ACN: 0.1 M TEAA,
1 M ammonium chloride, pH 8.5

Gradient: 0-40% B in 10 min, followed by
40-70% B in 14 min and
70-100% B in 25 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 220 nm

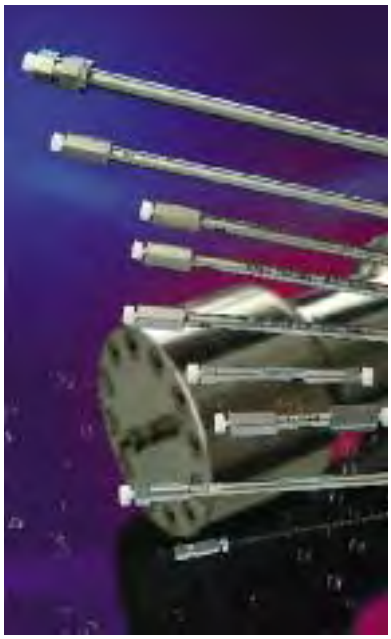


PL-SAX Strong Anion-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SAX 1000Å	PL-SAX 4000Å
1.0 x 50	5	207 bar, 3000 psi	PL1351-1502	PL1351-1503
2.1 x 50	5	207 bar, 3000 psi	PL1951-1502	PL1951-1503
4.6 x 50	5	207 bar, 3000 psi	PL1551-1502	PL1551-1503
2.1 x 50	8	207 bar, 3000 psi	PL1951-1802	PL1951-1803
2.1 x 150	8	207 bar, 3000 psi	PL1951-3802	PL1951-3803
4.6 x 50	8	207 bar, 3000 psi	PL1551-1802	PL1551-1803
4.6 x 150	8	207 bar, 3000 psi	PL1551-3802	PL1551-3803
4.6 x 250	10	207 bar, 3000 psi	PL1551-5102	PL1551-5103
4.6 x 150	10	207 bar, 3000 psi	PL1551-3102	PL1551-3103
25 x 50	10	207 bar, 3000 psi	PL1251-1102	PL1251-1103
25 x 150	10	207 bar, 3000 psi	PL1251-3102	PL1251-3103
50 x 150	10	207 bar, 3000 psi	PL1751-3102	PL1751-3103
100 x 300	10	207 bar, 3000 psi	PL1851-2102	PL1851-2103
4.6 x 250	30	207 bar, 3000 psi	PL1551-5702	PL1551-5703
4.6 x 150	30	207 bar, 3000 psi	PL1551-3702	PL1551-3703
25 x 150	30	207 bar, 3000 psi	PL1251-3702	PL1251-3703
50 x 150	30	207 bar, 3000 psi	PL1751-3702	PL1751-3703
100 x 300	30	207 bar, 3000 psi	PL1851-3102	PL1851-3103

PL-SAX Strong Anion-Exchange Bulk Media

Size	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å
100 g	10	PL1451-4102	PL1451-4103
1 kg	10	PL1451-6102	PL1451-6103
100 g	30	PL1451-4702	PL1451-4703
1 kg	30	PL1451-6702	PL1451-6703



PL-SCX Strong Cation-Exchange Columns

- Optimal design for effective separation of biomolecules
- Pore sizes allow use of a range of solute sizes
- Exceptional stability for long column lifetime

PL-SCX -SO_3^- is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation and purification of a wide range of biomolecules, from small peptides to large proteins. Two pore sizes are available, 1000Å and 4000Å, to provide good mass transfer characteristics for a range of solute sizes. The 5 µm media delivers separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.

Column Specifications

Bonded Phase	ID (mm)	Particle Size (µm)	Pore Size	pH Stability	Operating Temperature Limit
Strong Cation-Exchange	2.1, 4.6, 7.5, 25, 50 and 100	5, 8, 10 and 30	1000Å and 4000Å	1-14	80 °C

Standard protein separation

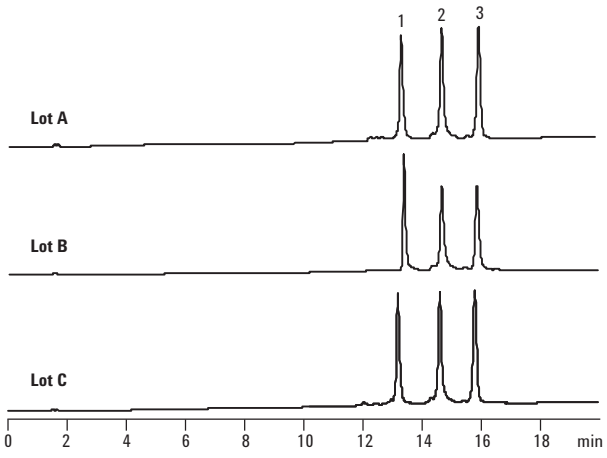
Column: **PL-SCX 1000Å**
PL1545-1502
4.6 x 50 mm, 5 µm

Mobile Phase: A: 20 mM KH_2PO_4, pH 6.0
B: A + 1 M NaCl

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Myoglobin
2. Chymotrypsinogen A
3. Cytochrome c
4. Lysozyme

PL-SCX Strong Cation-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SCX 1000Å	PL-SCX 4000Å
1.0 x 50	5	207 bar, 3000 psi	PL1345-1502	PL1345-1503
2.1 x 50	5	207 bar, 3000 psi	PL1945-1502	PL1945-1503
4.6 x 50	5	207 bar, 3000 psi	PL1545-1502	PL1545-1503
2.1 x 50	8	207 bar, 3000 psi	PL1945-1802	PL1945-1803
2.1 x 150	8	207 bar, 3000 psi	PL1945-3802	PL1945-3803
4.6 x 50	8	207 bar, 3000 psi	PL1545-1802	PL1545-1803
4.6 x 150	8	207 bar, 3000 psi	PL1545-3802	PL1545-3803
4.6 x 150	10	207 bar, 3000 psi	PL1545-3102	PL1545-3103
4.6 x 250	10	207 bar, 3000 psi	PL1545-5102	PL1545-5103
25 x 50	10	207 bar, 3000 psi	PL1245-1103	PL1245-1103
25 x 150	10	207 bar, 3000 psi	PL1245-3103	PL1245-3103
50 x 150	10	207 bar, 3000 psi	PL1745-3103	PL1745-3103
100 x 300	10	207 bar, 3000 psi	PL1845-2103	PL1845-2103
4.6 x 150	30	207 bar, 3000 psi	PL1545-3702	PL1545-3703
4.6 x 250	30	207 bar, 3000 psi	PL1545-5703	PL1545-5703
25 x 150	30	207 bar, 3000 psi	PL1245-3702	PL1245-3703
50 x 150	30	207 bar, 3000 psi	PL1745-3703	PL1745-3703
100 x 300	30	207 bar, 3000 psi	PL1845-3102	PL1845-3103

PL-SCX Strong Cation-Exchange Bulk Media

Size	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å
100 g	10	PL1445-4102	PL1445-4102
1 kg	10	PL1445-6102	PL1445-6103
100 g	30	PL1445-4702	PL1445-4703
1 kg	30	PL1445-6702	PL1445-6703

Bio-Monolith Ion-Exchange HPLC Column

Agilent Bio-Monolith Ion-Exchange HPLC Columns

- Polymer-based, monolith HPLC columns designed for macro biomolecule separations
- Flow-rate independent separations; no diffusion, no pores and no void volume make transport between mobile and stationary phase very rapid
- Monolith disk is 5.2 mm x 4.95 mm (100 µL column volume) with continuous channels, eliminating diffusion mass transfer
- Extremely fast separations speed up method development time and decrease costs; locking in method parameters takes significantly less time and buffer

Agilent Bio-Monolith Ion-Exchange HPLC columns provide high resolution and rapid separations of antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro biomolecules. The product family offers strong cation-exchange, strong and weak anion-exchange and Protein A phases. Bio-Monolith HPLC columns are compatible with HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems.

Agilent Bio-Monolith HPLC Column Selection Guide

Column	Description	Key Applications	Part No.
Bio-Monolith QA	The quaternary amine bonded phase (Strong Anion-Exchange) is fully charged over a working pH range of 2-13, binding negatively charged biomolecules.	• Adenovirus process monitoring and quality control • IgM purification monitoring and quality control • Monitoring DNA impurity removal • Monitoring endotoxin removal • HSA Purity	5069-3635
Bio-Monolith DEAE	The diethylaminoethyl bonded phase (Weak Anion-Exchange) offers increased selectivity of biomolecules with negative charge over a working pH range of 3-9.	• Process monitoring and quality control of bacteriophage manufacturing and purification • Process monitoring and quality control of plasmid DNA purification	5069-3636
Bio-Monolith SO_3	The sulfonyl bonded phase (Strong Cation-Exchange) is fully charged over a working pH range of 2-13, binding positively charged biomolecules.	• Fast and high resolution analytical separations of large molecules such as proteins and antibodies • Hemoglobin A1c fast analytics	5069-3637

TIPS & TOOLS

Agilent also offers a Protein A Bio-Monolith column for affinity chromatography. For more information, see pages 434-436.

Column Specifications	
Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2200 psi)
Temperature min/max	Working: 4-40 °C
	Storage: 4-30 °C
Recommended pH	Working range: 2-13
	Cleaning-in-place: 1-14
Materials of construction	Hardware: Stainless steel
	Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith QA: Blue
	Bio-Monolith DEAE: Green
	Bio-Monolith SO_3: Red
Shelf life/expiration date	SO_3, QA, DEAE: 24-36 months

Baseline expansion of a separation of protein standards

Column: **Agilent Bio-Monolith CM15, 5.5 x 15 mm**

Mobile Phase: A: 10 mM Na_2HPO_4, pH 6.0
B: A + 0.5 M NaCl or just 0.5 M Na_2HPO_4, pH 6.0

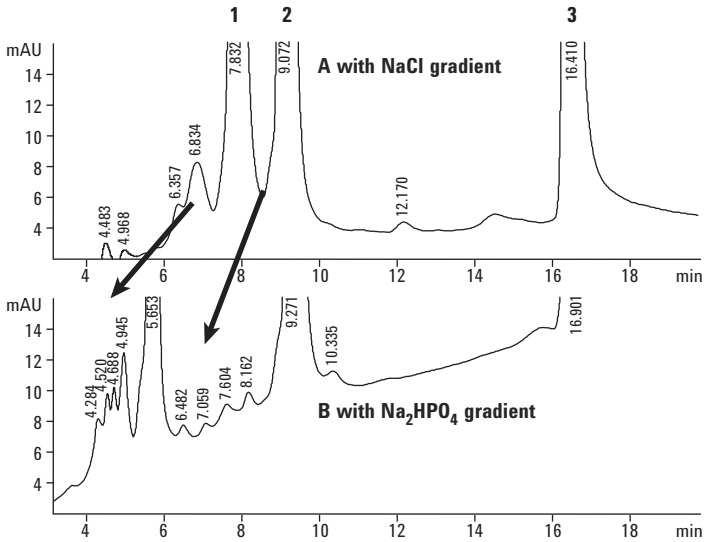
Flow Rate: 2 mL/min

Gradient: 0.5 min hold with mobile phase A followed by a linear gradient to 45% B in 15 min (elapsed time 15.5 min); then 60% B at 15.6 min continued to 20 min. Column flushed with 100% B for 15 min before re-equilibration for the next run.
pH Gradient: A: 5 mM Na_2HPO_4, buffer pH 5.5 and B: 40 mM NA_2HPO_4 (not buffered, pH 8.9). 2% B/min at 1 mL/min for 15 min, followed by a column wash with 90% B for 5 min.

Detector: UV at 220 nm

Sample: One mg each/mL in mobile phase A.
1. RNAse from bovine pancreas (pI 9.6)
2. Cytochrome c from bovine heart (pI 10.37-10.8)
3. Lysozyme from chicken egg (pI 11.35) (0.5 mg)

Instrument: Agilent 1200 SL with diode array detector



B shows a better resolution of protein contaminants.

Bio-Monolith DEAE column monitors phage production during fermentation

Column: **DEAE**
5069-3636
5.2 x 4.95 mm

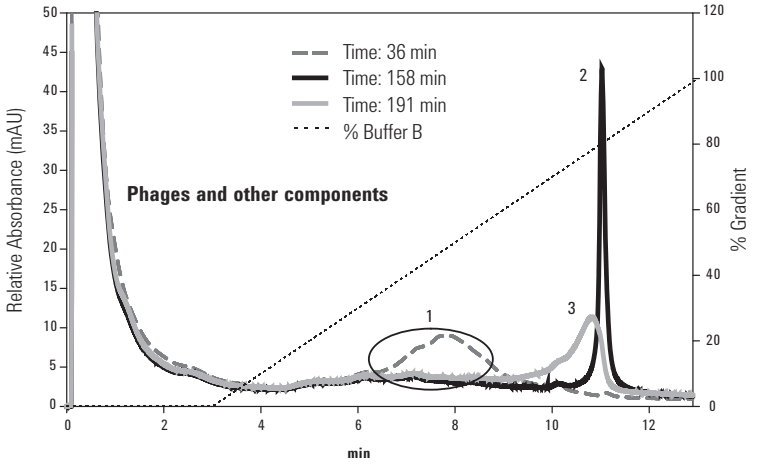
Mobile Phase: A: 125 mM Phosphate buffer, pH 7.0
B: 125 mM Phosphate buffer + 1 M NaCl, pH 7.0

Flow Rate: 1 mL/min

Gradient: 100% buffer A (2.5 min)
0-100% buffer B (10 min)
100% buffer A (2 min)

Detector: UV at 280 nm

Instrument: High pressure gradient HPLC system, Agilent 1200 Infinity LC



As phage proliferation progresses, the genomic DNA (gDNA) concentration increases as the host cells are being lysed. In the late stages of fermentation, gDNA begins to degrade into fragments. These gDNA fragments cannot be easily removed by purification media, therefore it is critical to stop the fermentation cycle prior to the degradation of the genomic DNA. The chromatogram above represents three samples taken from the bioreactor at 36, 158 and 191 minutes. Peak 1 represents phage, media and host cells, peak 2 the intact gDNA and peak 3 the fragmented gDNA.

Size Exclusion Chromatography (SEC)

Accurately determine biomolecule aggregation, fragmentation, and chemical ligation/modification

Size exclusion chromatography (SEC) is a technique for separating proteins, oligonucleotides, and other complex biopolymers by size using aqueous eluents.

Applying SEC to aggregation studies

The size, type, and content of aggregates present in protein biopharmaceuticals can affect both efficacy and formulation – or worse, induce an immunogenic response. Aggregation formations occur through a variety of mechanisms, including disulfide bond formation and non-covalent interactions.

Because the size of protein aggregates, including dimers, is sufficiently different from the protein monomer, you can separate the various forms using SEC. In fact, SEC with UV or light scattering is a standard technique for quantifying protein aggregation.

Applying SEC to quantitation and molecular weight determination

For proteins and other molecules of discreet molecular weight, SEC can be used to detect and quantitate monomers, dimers, aggregates and fragments. SEC can also separate oligonucleotide mixtures.

For biopolymers of varying sizes, like starches and other polysaccharides, SEC can provide data on molecular weight distribution and branching (with the proper detectors).

As a leading manufacturer of SEC columns and instruments for over 30 years, Agilent is continually developing new SEC products that will provide even higher resolution and quicker separations. This section highlights Agilent's broad family of SEC columns for protein biopolymer analysis:

- **Bio SEC-3 and Bio SEC-5 columns** are available in a variety of pore sizes, and are well suited for protein analysis – especially when determining the presence of dimers and aggregates in therapeutic biologicals. Note that 3 µm Bio SEC-3 columns provide higher resolution than our industry-standard 5 µm Bio SEC-5 columns.
- **ProSec 300S columns** work well with globular proteins under high salt conditions.
- **ZORBAX GF-250 and GF-450 columns** are best for preparative SEC of proteins, because of their larger column size and higher flow rates.
- **PL aquagel-OH columns** can be used to analyze biopolymers of broad molecular weights, such as PEGs, oligo- and polysaccharides, starches, and gums.

Size Exclusion Chromatography (SEC)

Application	Agilent Columns	Notes
Peptides, proteins	Agilent Bio SEC-3	Higher resolution and faster separations from 3 µm particles, with 100Å, 150Å, and 300Å pore sizes.
Large biomolecules and samples with multiple molecular weight components	Agilent Bio SEC-5	More pore size options (100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å) to cover a wider range of analytes.
Globular proteins, antibodies	ProSEC 300S	Single column option for protein analysis in high salt conditions.
Proteins, globular proteins	ZORBAX GF-250/450	Higher flow rate capabilities and larger column size for SEC semi-prep and prep.
Low MW polymers and oligomers, oligosaccharides, PEGs, lignosulfonates	2 or 3 PL aquagel-OH • PL aquagel-OH 8 µm • PL aquagel-OH 20 5 µm • PL aquagel-OH MIXED-M 8 µm	The PL aquagel-OH analytical series has a pH range of 2-10, compatibility with organic solvent (up to 50% methanol), mechanical stability up to 140 bar (2030 psi), and low column operating pressures.
Polydisperse biopolymers, polysaccharides, cellulose derivatives	2 or 3 PL aquagel-OH • PL aquagel-OH MIXED-H 8 µm • PL aquagel-OH 60/50/40 8 µm	
Very high MW polymers, hyaluronic acids, starches, gums	PL aquagel-OH 60/50/40 15 µm in series	

Agilent Bio SEC-3

- Exceptional loading capacity, stability, and reproducibility for size-based biomolecule separations
- Sharper peaks, higher resolution, and better protein recovery
- Faster separations than large-particle SEC columns
- Compatibility with most aqueous buffers
- Excellent stability in high-salt and low-salt conditions

Agilent Bio SEC-3 HPLC columns are a breakthrough technology for size exclusion chromatography (SEC). They are packed with spherical, narrowly dispersed 3 µm silica particles coated with a proprietary hydrophilic layer. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient size exclusion particle.

Agilent Bio SEC-3 HPLC columns are available in 100Å, 150Å and 300Å pore sizes to accommodate most peptide and protein size exclusion separations.

Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	3 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	3 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	3 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

TIPS & TOOLS

Deactivated/silanized vials have inert surfaces that will not interact with metals, biologicals or proteins, and will not cause pH shifts. Avoid standard polypropylene vials for biological or light-sensitive compounds.

Calibration curves – Bio SEC-3

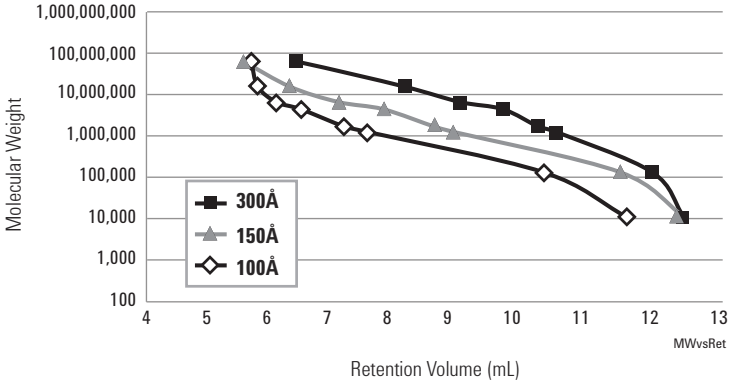
Column: **Bio SEC-3**
7.8 x 300 mm, 3 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

Detector: UV

Proteins	MWt	300Å	150Å	100Å
Thyroglobulin	670000	6.34	5.50	5.63
Gamma globulin	158000	8.03	6.24	5.74
BSA	67000	8.90	7.00	6.03
Ovalbumin	45000	9.57	7.70	6.41
Myoglobin	17000	10.12	8.50	7.10
Ribonuclease A	12700	10.40	8.80	7.46
Vitamin B-12	1350	11.90	11.40	10.20



Intact MAb monomer and dimer separation

Column: **Bio SEC-3, 300Å**
5190-2511
7.8 x 300 mm, 3 µm

Buffer: Sodium phosphate buffer, pH 7.0, 150 mM

Isocratic: 0-100% Buffer A from 0-30 min

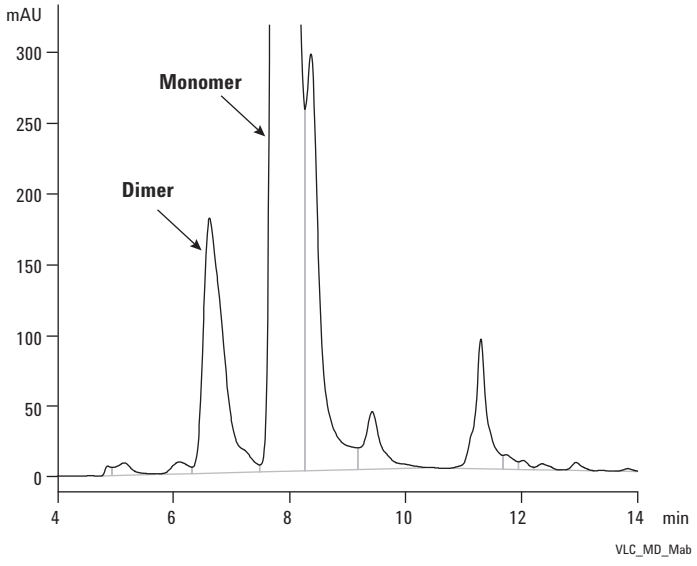
Flow Rate: 1.0 mL/min

Sample: CHO-humanized MAb, 5 mg/mL – intact

Injection: 5 µL

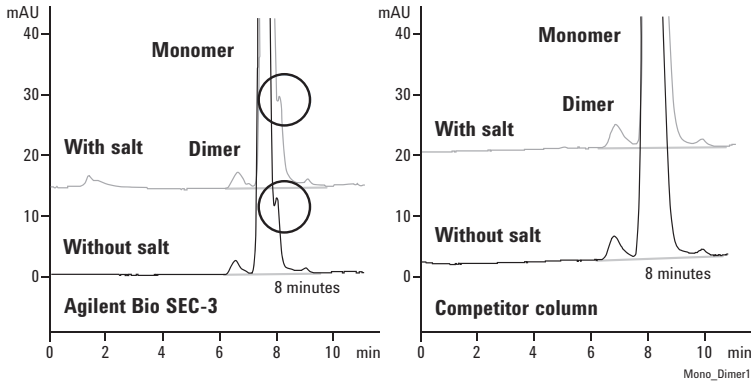
Detector: UV 220 nm

Temperature: Ambient



Comparison of Agilent Bio SEC-3 and competitor column in the analysis of a monoclonal antibody

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Column:	Competitor 7.8 x 300 mm
Mobile Phase:	150 mM sodium phosphate + 100 mM Na sulfate (with salt) 150 mM sodium phosphate (without salt)
Flow Rate:	1.0 mL/min
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



The Agilent Bio SEC-3 column reveals the presence of smaller MW species missed by the competitor column.

Monoclonal Antibody Monomer and Dimer Analysis using Agilent Bio SEC-3 and a Competitor Column

Eluent	Column	Resolution Ratio Monomer:Dimer	Monomer Efficiency	Percentage Dimer
With salt	Agilent	2.04	7,518	0.59
With salt	Competitor	1.88	3,967	0.59
Without salt	Agilent	2.08	7,942	0.60
Without salt	Competitor	1.92	4,164	0.57

Pore Size Choice

The choice of media pore size will influence the resolution in SEC. As the separation is based on differences in molecular size in solution, the sample must be able to permeate the porous structure of the particles – if the pore size is too small, the samples will be excluded from the pores and elute in the void volume of the column, and if too large then, all will be able to fully permeate the particles and so there will be very little separation.

Pore size choice: Proteins

Column A: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 150Å**
5190-2508
4.6 x 300 mm, 3 µm

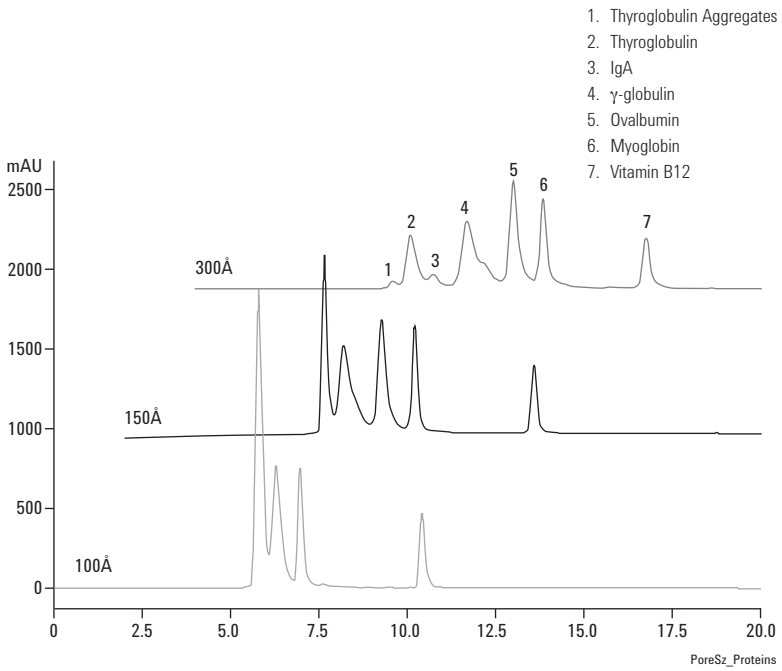
Column C: **Bio SEC-3, 300Å**
5190-2513
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 + 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV, 220 nm

Sample: BioRad Gel Filtration Standards Mix



Pore size choice: Mouse IgG

Column A: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 150Å**
5190-2508
4.6 x 300 mm, 3 µm

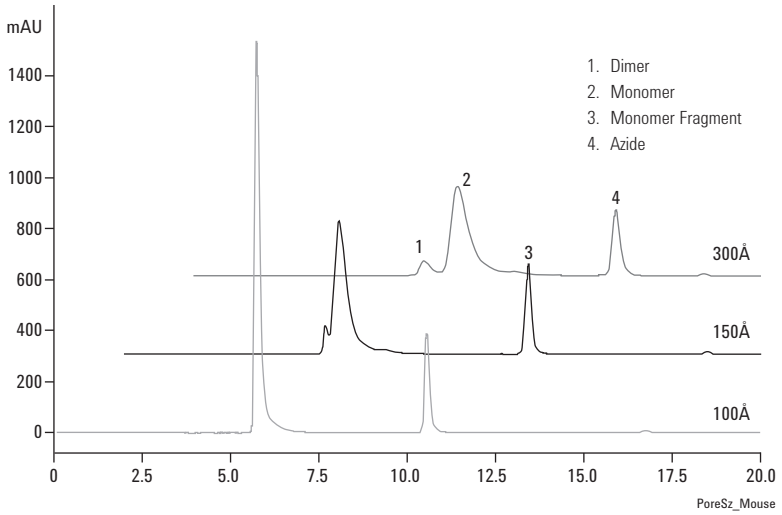
Column C: **Bio SEC-3, 300Å**
5190-2513
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 + 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV, 220 nm

Sample: Mouse IgG

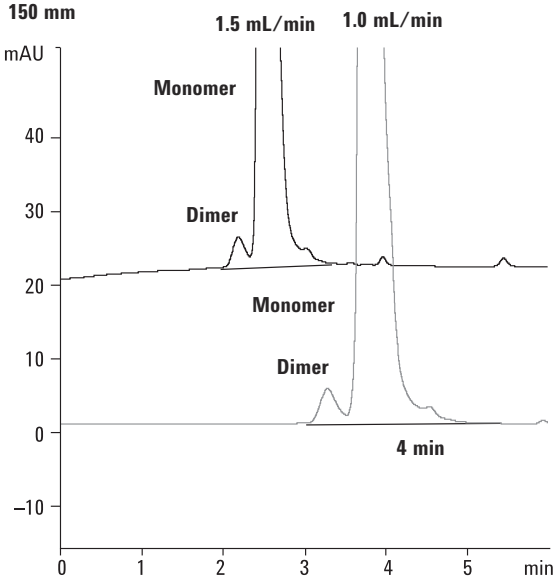


Column Length

Where the separation time is a critical parameter, shorter columns packed with the higher efficiency, 3 µm media are used. With the shorter columns, higher flow rates are used to reduce the analysis time but without compromising the quality of the data – quantitation of monoclonal antibody monomer and dimer.

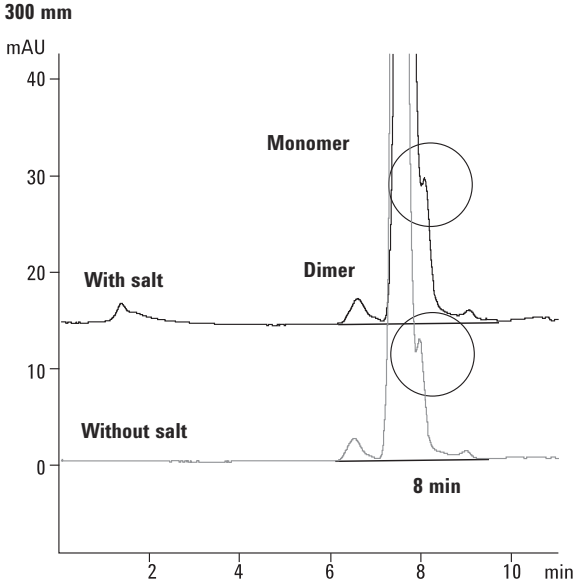
Agilent Bio SEC-3 column length comparison, 150 mm

Column:	**Bio SEC-3, 300Å** **5190-2512** **7.8 x 150 mm, 3 µm**
Mobile Phase:	150 mM sodium phosphate
Flow Rate:	1.0 mL/min (56 bar), 1.5 mL/min (75 bar)
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



Agilent Bio SEC-3 column length comparison, 300 mm

Column:	**Bio SEC-3, 300Å** **5190-2511** **7.8 x 300 mm, 3 µm**
Mobile Phase:	150 mM sodium phosphate + 100 mM Na sulfate (with salt) 150 mM sodium phosphate (without salt)
Flow Rate:	1.0 mL/min
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



Agilent Bio SEC-3

Size (mm)	Particle Size (µm)	Bio SEC-3 100Å USP L33	Bio SEC-3 150Å USP L33	Bio SEC-3 300Å USP L33
7.8 x 300	3	5190-2501	5190-2506	5190-2511
7.8 x 150	3	5190-2502	5190-2507	5190-2512
4.6 x 300	3	5190-2503	5190-2508	5190-2513
4.6 x 150	3	5190-2504	5190-2509	5190-2514
7.8 x 50, Guard	3	5190-2505	5190-2510	5190-2515

Agilent Bio SEC-5

- Maximum recovery for a broad range of size-based, biomolecule separations
- Outstanding reproducibility and column lifetime
- Excellent stability, even under high-pH, high-salt, and low-salt conditions
- Compatibility with most aqueous buffers

Agilent Bio SEC-5 HPLC columns are packed with 5 µm silica particles coated with a proprietary, neutral, hydrophilic layer for maximum efficiency and stability. Our specially designed packing also provides high pore volume, improving both peak capacity and resolution.

Bio SEC-5 columns are available in 5 µm particles with 100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å nominal pore sizes.

Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	5 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	5 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	5 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
500Å	5 µm	15,000-5,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
1000Å	5 µm	50,000-7,500,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
2000Å	5 µm	>10,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

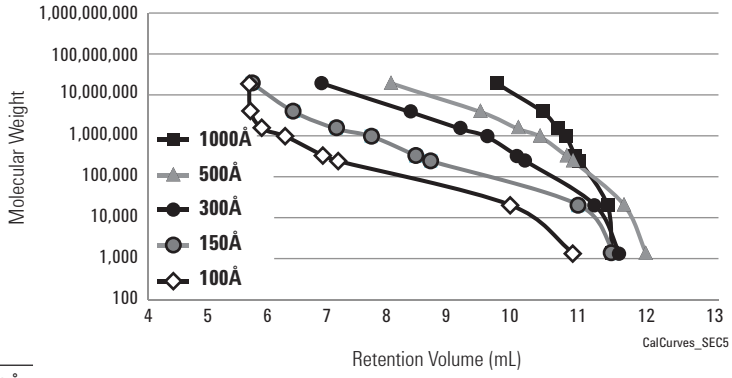
Calibration curves – Bio SEC-5

Column: **Bio SEC-5**
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

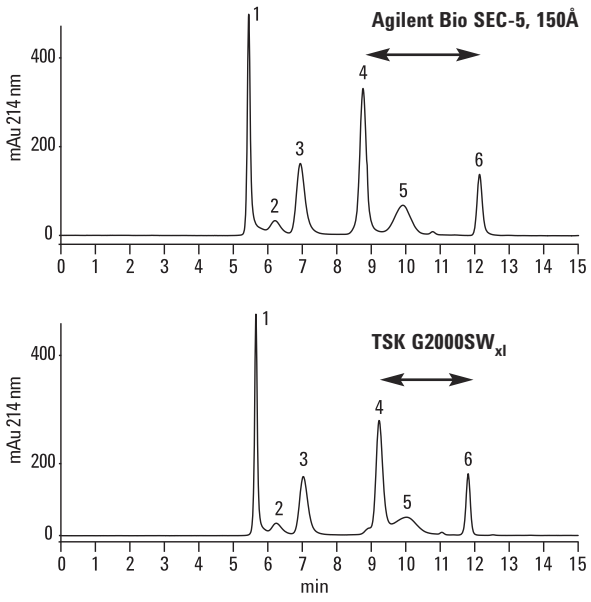
Flow Rate: 1.0 mL/min

Detector: UV, 214 nm



		Retention Volume				
Proteins	**MW**	**1000Å**	**500Å**	**300Å**	**150Å**	**100Å**
Thyroglobulin	670000	10.07	8.23	7.03	5.82	5.77
Gamma globulin	158000	10.88	9.80	8.57	6.55	5.79
BSA	67000	11.13	10.44	9.44	7.29	6.00
Ovalbumin	45000	11.28	10.83	9.89	7.90	6.40
Myoglobin	17000	11.44	11.28	10.42	8.66	7.05
Ribonuclease A	12700	11.52	11.41	10.58	8.93	7.32
Vitamin B-12	1350	12.00	12.59	11.78	11.49	10.30

Side-by-side comparison



Column: **Bio SEC-5**
5190-2521
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

Detector: UV, 214 nm

1. Thyroglobulin, 5.43 min
2. BSA dimer, 6.19 min
3. BSA monomer, 6.93 min
4. Ribonuclease A, 8.74 min
5. Poly-DL-alanine (1-5 kDa), 9.90 min
6. Uracil, 12.13 min

1. Thyroglobulin, 5.64 min
2. BSA dimer, 6.23 min
3. BSA monomer, 7.02 min
4. Ribonuclease A, 9.22 min
5. Poly-DL-alanine (1-5 kDa), 10.02 min
6. Uracil, 11.81 min

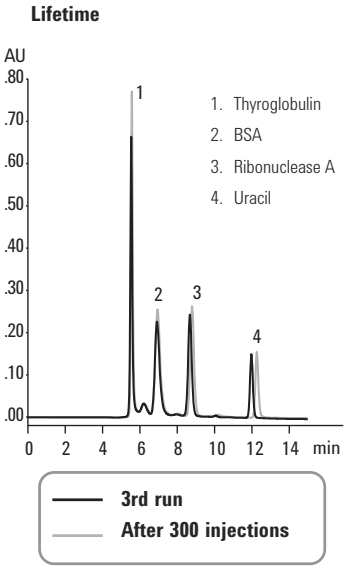
Separation of a protein mixture on an Agilent Bio SEC-5 HPLC column and a Tosoh TSK-Gel column. Notice the sharper peaks and better resolution on the Agilent Bio SEC-5 HPLC column.

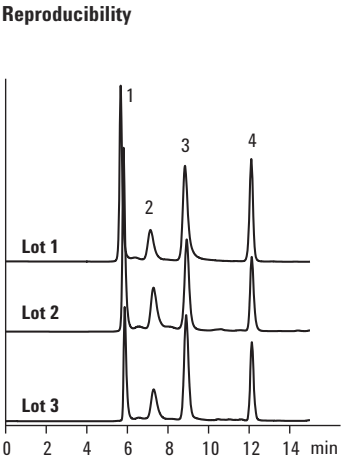
Exceptional lifetime, and lot-to-lot reproducibility

Column: **Bio SEC-5, 150Å**
5190-2521
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Phosphate Buffer, pH 7.0

The four protein mixture shows excellent retention time reproducibility over 300 injections and on three columns from different manufacturing lots.





Comparison between Agilent Bio SEC-3 and Agilent Bio SEC-5

Analysis of monoclonal antibody

Column: **Bio SEC-3, 300Å**
5190-2511
7.8 x 300 mm, 3 µm

Column: **Bio SEC-5, 300Å**
5190-2526
7.8 x 300 mm, 5 µm

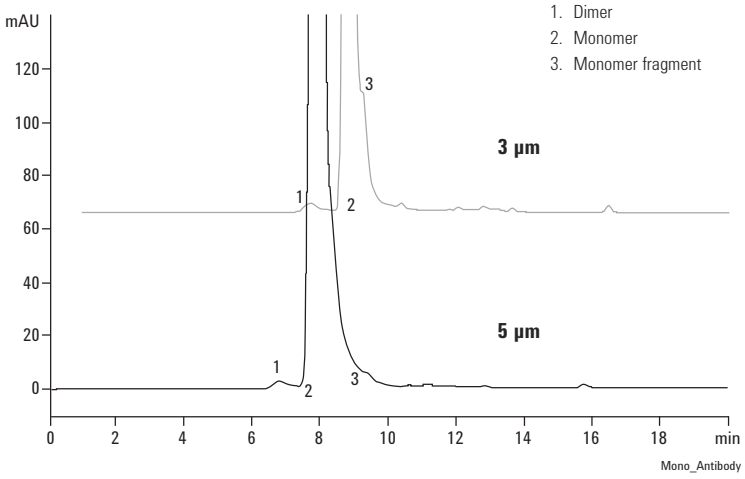
Mobile Phase: 150 mM Sodium Phosphate, pH 7

Flow Rate: 1 mL/min

Detector: UV @ 220 nm

Sample: Humanized monoclonal antibody

The 3 µm column gives better separation



Agilent Bio SEC-5

Size (mm)	Particle Size (µm)	Bio SEC-5 100Å USP L33	Bio SEC-5 150Å USP L33	Bio SEC-5 300Å USP L33	Bio SEC-5 500Å USP L33	Bio SEC-5 1000Å USP L33	Bio SEC-5 2000Å USP L33
7.8 x 300	5	5190-2516	5190-2521	5190-2526	5190-2531	5190-2536	5190-2541
7.8 x 150	5	5190-2517	5190-2522	5190-2527	5190-2532	5190-2537	5190-2542
4.6 x 300	5	5190-2518	5190-2523	5190-2528	5190-2533	5190-2538	5190-2543
4.6 x 150	5	5190-2519	5190-2524	5190-2529	5190-2534	5190-2539	5190-2544
7.8 x 50, Guard	5	5190-2520	5190-2525	5190-2530	5190-2535	5190-2540	5190-2545

TIPS & TOOLS

The Agilent rack can be used to optimize your 1290 Infinity LC for ultra-low dispersion, which can enhance performance of high-efficiency columns. Further information can be found in application note 5990-9502EN at **www.agilent.com/chem/library**

ProSEC 300S

- Mechanically robust silica particles that do not bleed during use
- Single column with extended linear resolving range
- Column dimensions for use with multi-detector systems

The Agilent ProSEC 300S column is specifically designed as a single column solution for globular protein analysis. The pore size selection and optimization provides an extended linear resolving range so that this single column can be used for analysis across the full range of globular proteins.

The particles are extremely robust and do not fragment during use to leach particulates. This gives exceptionally stable baselines making this column an ideal choice for use with light scattering detectors.

Two column dimensions, 7.5 mm id and 4.6 mm id, to suit multi-detector size exclusion chromatography provide an option for the analysis of small masses.

ProSEC 300S Column Specifications

Bonded Phase	Pore Size	Particle Size	Protein MW Range	pH Range	Flow Rate	Max Pressure
ProSEC 300S	300Å	5 µm	1,500-800,000	2-7.5	<1.5 mL/min (7.5 mm id)	250 bar, 3700 psi
					<0.5 mL/min (4.6 mm id)	

ProSEC 300S

Dimensions	Particle Size (µm)	Part No.
4.6 x 250	5	PL1547-5501
7.5 x 300	5	PL1147-6501
Guard Columns		
4.6 x 50	5	PL1547-1501
7.5 x 50	5	PL1147-1501

Calibration of the ProSEC 300S column with globular proteins

Mobile Phase: 50 mM KH_2PO_4-K_2HPO_4 (@ pH 6.8) containing 0.3 M NaCl

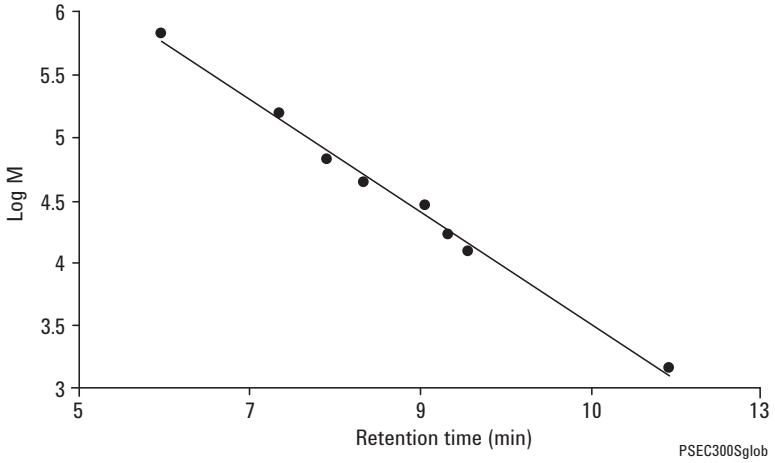
Flow Rate: 1.0 mL/min

Detector: UV, 280 nm

Sample: Protein samples

Molecular weights of the proteins

Mw/Daltons	Protein
670,000	Thyroglobulin
155,000	γ-Globulin
66,430	Bovine serum albumin
44,287	Ovalbumin
29,000	Carbonic anhydrase
16,700	Myoglobulin
12,384	Cytochrome c
1,423	Bacitracin



Analysis of Bovine Serum Albumin by light scattering using ProSEC 300S columns

Column: ProSEC 300S
PL1147-6501
7.5 x 300 mm, 5 µm

Mobile Phase: Water + 120 mM NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4

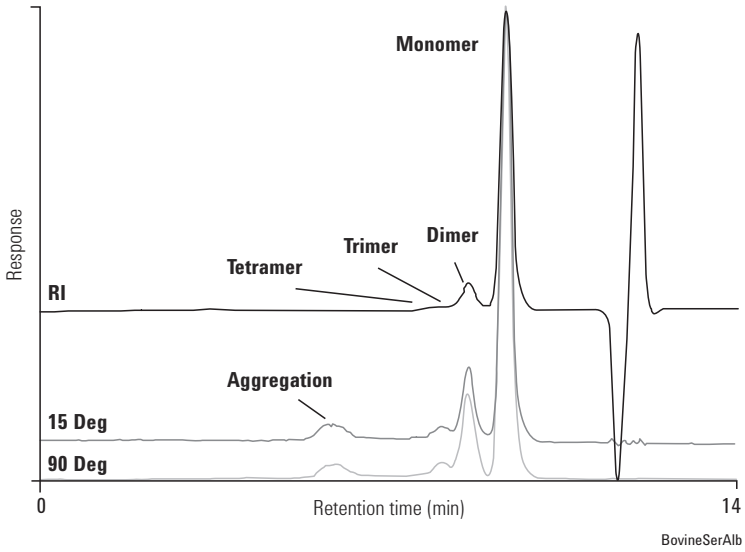
Flow Rate: 1.0 mL/min

Detector: Differential refractive index + PL-GPC 50 Dual Angle Light Scattering Detector

Sample: Bovine serum albumin

Molecular Weights

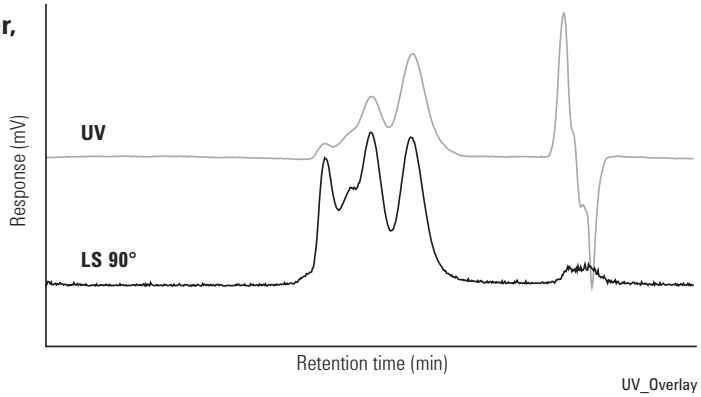
Monomer	66,900 Daltons, 88.5%
Dimer	34,900 Daltons (2.02 x monomer molecular weight), 9.8%
Trimer	197,000 Daltons (2.94 x monomer molecular weight), 1.2%
Tetramer	279,300 Daltons (5.17 x monomer molecular weight), 0.5%



Overlay of differential refractive index and dual angle light scattering sample.

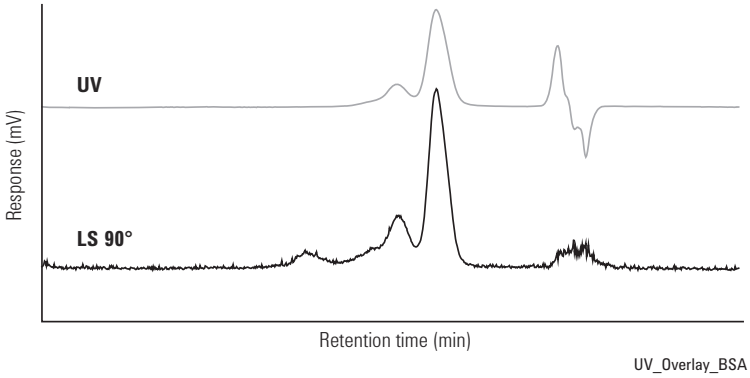
Overlay of UV and light scattering 90° for a sample of γ-globulins, illustrating monomer, dimer, and trimer peaks

Column:	**ProSEC 300S PL1147-6501 7.5 x 300 mm, 5 µm**
Mobile Phase:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0
Flow Rate:	1.0 mL/min
Temperature:	5 °C
Detector:	UV at 310 nm + PL-GPC 50 Dual Angle Light Scattering Detector
Sample:	Proteins



Overlay of UV and light scattering 90° for a sample of BSA, illustrating monomer, dimer, trimer and aggregate peaks

Column:	**ProSEC 300S PL1147-6501 7.5 x 300 mm, 5 µm**
Mobile Phase:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0
Flow Rate:	1.0 mL/min
Temperature:	5 °C
Detector:	UV at 310 nm + PL-GPC 50 Dual Angle Light Scattering Detector
Sample:	Proteins



ZORBAX GF-250 and GF-450 Gel Filtration Columns

GF-250 Gel Filtration Columns

- High efficiency and reproducibility with short analysis time
- Semi-prep and prep column dimensions
- Compatible with organic modifiers and denaturants
- Wide usable pH range (3-8)

Agilent ZORBAX GF-250 and GF-450 size exclusion (gel filtration) columns are ideal for 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 for a pH operating 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 that can be used for both analytical and preparative 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 reduce protein aggregation. 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

Specifications represent typical values only

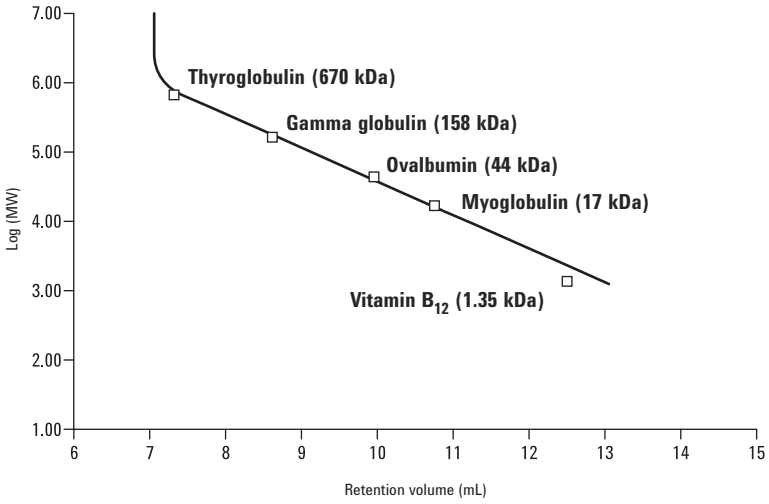
Retention volume versus log (MW) for the Bio-Rad standards separated on an Agilent ZORBAX GF-250 column

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

Mobile Phase: 200 mM Sodium phosphate, pH 7.0

Temperature: Ambient

Detector: UV, 254 nm



Separations of proteins on preparative columns

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

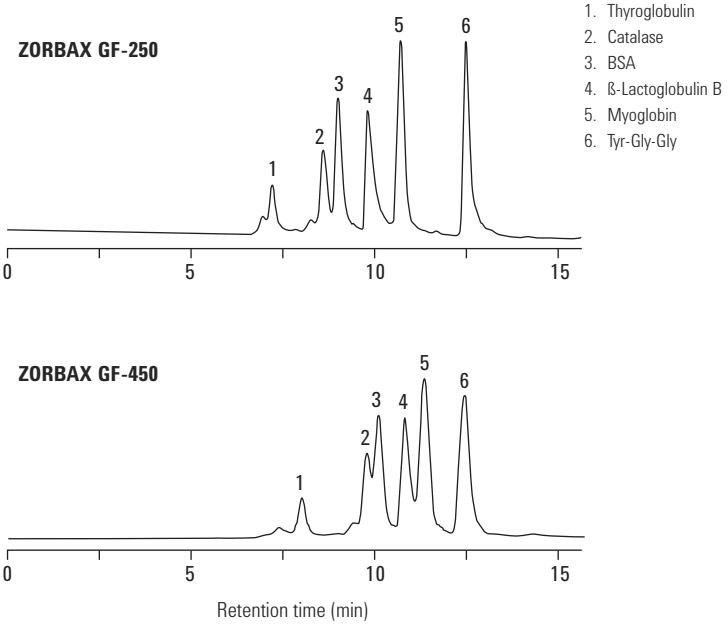
Column: **ZORBAX GF-450**
884973-902
9.4 x 250 mm, 6 µm

Mobile Phase: 0.2 M Na_2HPO_4, pH 7.0

Flow Rate: 5.0 mL/min

Detector: UV, 280 nm

Sample: 200 µL



ZORBAX GF-250 (USP L33) and GF-450 (USP L35) 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)				
P	GF-250 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
ZGC	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
P	GF-450 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
ZGC	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
P	Prep Guard Hardware Kit			840140-901
ZGC	Guard Hardware Kit			820999-901
PrepHT Columns				
PL	PrepHT GF-250, 150Å	21.2 x 250	6	877974-901
PL	PrepHT GF-450, 300Å	21.2 x 250	6	877974-910
PL	PrepHT Endfittings, 2/pk			820400-901
PL	PrepHT GF-250, Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-911
PL	PrepHT GF-450, Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-911
PL	Guard Cartridge Hardware			820444-901

Bio-Monolith Protein A Column, 5069-3639

Affinity Chromatography

Affinity chromatography is a powerful technique which takes advantage of highly specific molecular interactions, frequently between specific proteins (e.g. antigen/antibody). Agilent offers several specialty affinity products, a monolithic Protein A column for the isolation and quantitation of IgG and a series of Multiple Affinity Removal Systems for the elimination of high abundance proteins in biological samples.

Agilent Bio-Monolith Protein A HPLC Columns

- Designed for the analytical separation of all IgG (human and mouse), except for IgG class3
- Flow-rate independent separations; no diffusion, no pores and no void volume make transport between mobile and stationary phase very rapid
- Extremely fast separations speed up method development time and decrease costs
- Locking in method parameters takes significantly less time and buffer

Agilent Bio-Monolith Protein A HPLC columns are part of the Agilent Bio-Monolith column family. Protein A Bio-Monolith columns are compatible with HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems.

TIPS & TOOLS

For information on Ion-Exchange Bio-Monolith columns, turn to pages 412-415.

Column Specifications	
Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2200 psi)
Temperature min/max	Working: 4-40 °C
	Storage: 4-30 °C
Recommended pH	Working range: 2-13
	Cleaning-in-place: 1-14
Materials of construction	Hardware: Stainless steel
	Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith Protein A: White
Shelf life/expiration date	Protein A: 12 months

Bio-Monolith Protein A

Column	Description	Key Applications	Part No.
Bio-Monolith Protein A	The Protein A affinity column is designed for the analytical separation of all IgG (human and mouse), except for IgG class3.	• Quantitative determination of IgG (fermentation titer calculation)	5069-3639

TIPS & TOOLS

Further information can be found in the following application note:

Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column (publication # 5989-9733EN)

www.agilent.com/chem/library

Rapid human polyclonal IgG quantification using the Agilent Bio-Monolith Protein A HPLC column

Column: **Protein A**
5069-3639
5.2 x 4.95 mm

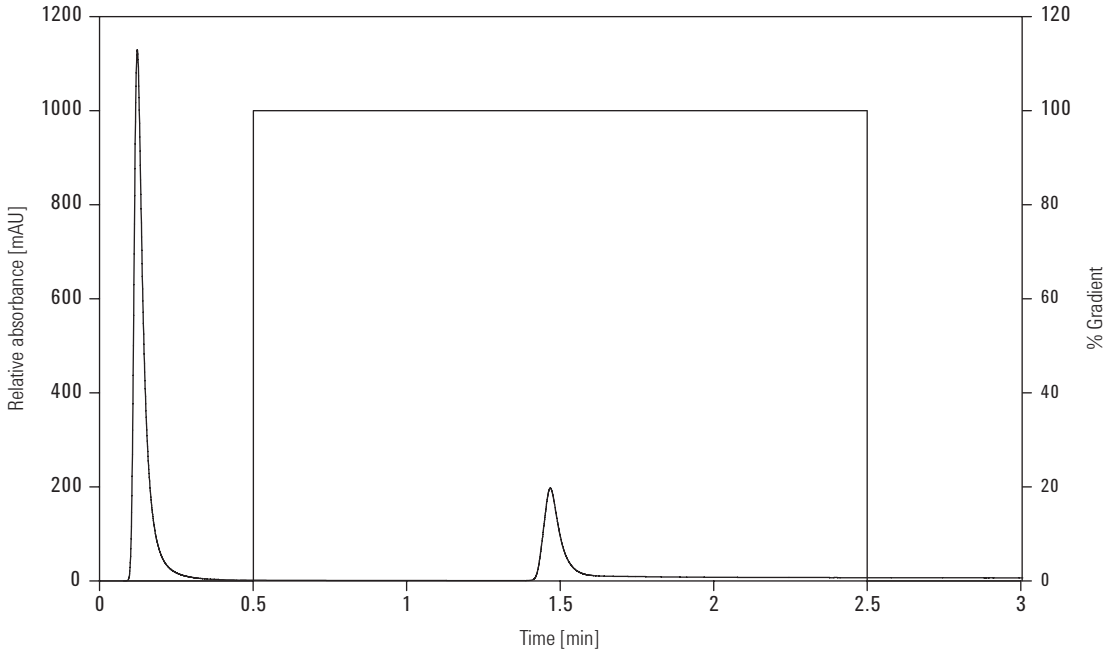
Mobile Phase: PBS buffer, pH 7.4
0.5 M acetic acid, pH 2.6

Flow Rate: 1 mL/min

Gradient: Stepwise gradient: 100% buffer A-100% buffer B-100% buffer A (0.5 min each step)

Detector: A high pressure gradient HPLC system, Agilent 1200 Infinity LC - UV at 280 nm

Sample: Human Plasma diluted with binding buffer (PBS buffer, pH 7.4)



The selectivity of the Bio-Monolith Protein A column for the IgG from human plasma. IgG binds to protein A, a 100% buffer B step gradient is applied, and IgG elutes at 1.5 min.

Key:

Lane 1: Whole serum prior to separation

Lane 2: IgG standard

Lane 3: Peak 1 (flow-through fraction)

Lane 4: Peak 2 (Protein A-bound fraction; i.e. IgG1 and IgG2)

SDS PAGE analysis of fractions from the separation.

Agilent Protein Fractionation System and Proteomics Reagents

- LC/MS analysis of biological samples
- Preparation for electrophoretic analysis
- Sample preparation for biomarker discovery
- Instrument and workflow validation
- Cost-effective immunodepletion
- Sample desalting, concentration, and fractionation

In order to more easily isolate and identify proteins in biological samples, such as serum, plasma, and cerebro-spinal fluid (CSF), the Agilent Multiple Affinity Removal System is designed to chromatographically eliminate interfering high-abundance proteins from biological samples. Removal of these abundant proteins improves the subsequent LC/MS and electrophoretic analysis of the sample by effectively expanding the dynamic range.

For sample fractionation and desalting, the Agilent mRP-C18 High-Recovery Protein column is designed to simultaneously desalt, concentrate, and fractionate in one easy step with extremely high recovery of samples as compared to conventional RP HPLC columns that are fully compatible with LC/MS analysis.

In addition, validated reagents for sample preparation in biomarker discovery and other proteomics applications are also available, including a complex standard, and proteomics grade trypsin. For your convenience, these reagents are fully compatible with Agilent LC/MS methods and require no additional sample pretreatments.

Large volume requirements and custom column dimensions can also be addressed with our custom configurations.

Multiple Affinity Removal System

Multiple Affinity Removal System

The Multiple Affinity Removal System from Agilent enables the identification and characterization of high-value, low abundant proteins and biomarkers found in serum, plasma, and other biological fluids.

The Multiple Affinity Removal System reproducibly and specifically removes up to 14 high abundant proteins found in human biological fluids and 3 high abundant proteins found in mouse biological fluids.

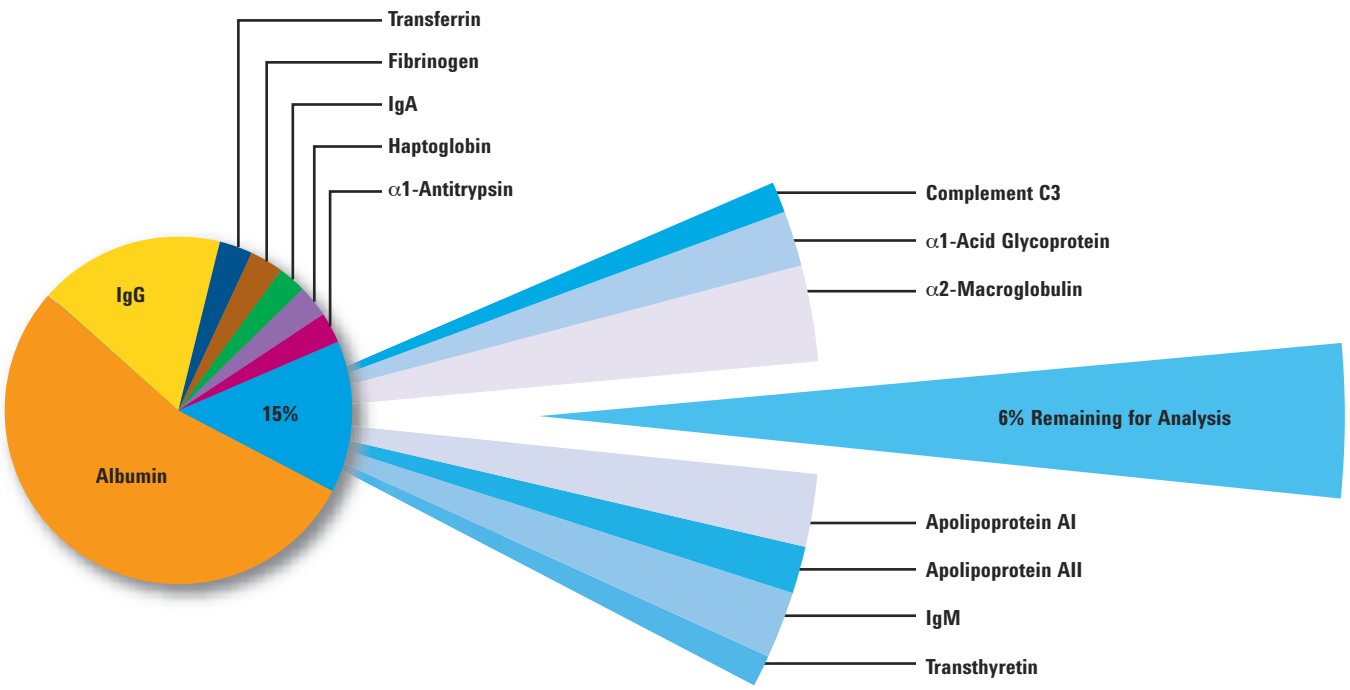
The Multiple Affinity Removal System is available in a variety of LC column dimensions and in spin cartridge format. When combined with Agilent's optimized buffers, convenient spin filters and concentrators, the Agilent Multiple Affinity Removal System creates an automated, integrated depletion solution compatible with most LC instruments (columns), and bench top centrifuges (spin cartridges).

Samples depleted using the Multiple Affinity Removal System are ready for downstream analyses such as 2-D gel electrophoresis, LC/MS, and other analytical techniques.

Multiple Affinity Removal System Selection Guide

Product	Proteins Removed	Total Protein Removed	Dimension	Load Capacity	Part No.
MARS Human-14	Albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin	94%	Spin Cartridge	8 - 10 µL	5188-6560
			4.6 x 50 mm	20 µL	5188-6557
			4.6 x 100 mm	40 µL	5188-6558
			10.0 x 100 mm	250 µL	5188-6559
MARS Human-7	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen	88-92%	Spin Cartridge	12 - 14 µL	5188-6408
			4.6 x 50 mm	30 - 35 µL	5188-6409
			4.6 x 100 mm	60 - 70 µL	5188-6410
			10.0 x 100 mm	250 - 300 µL	5188-6411
MARS Human-6	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	7 - 10 µL	5188-5230
			4.6 x 50 mm	15 - 20 µL	5185-5984
			4.6 x 100 mm	30 - 40 µL	5185-5985
MARS Human-6 High Capacity	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	14 - 16 µL	5188-5341
			4.6 x 50 mm	30 - 40 µL	5188-5332
			4.6 x 100 mm	60 - 80 µL	5188-5333
			10.0 x 100 mm	up to 340 µL	5188-5336
MARS Human-2	Albumin, IgG	69%	Spin Cartridge	50 µL	5188-8825
			4.6 x 50 mm	100 µL	5188-8826
MARS Human-1	Albumin	50-55%	Spin Cartridge	65 µL	5188-5334
			4.6 x 50 mm	130 µL	5188-6562
MARS Mouse-3	Albumin, IgG, transferrin	80%	Spin Cartridge	25 - 30 µL	5188-5289
			4.6 x 50 mm	37 - 50 µL	5188-5217
			4.6 x 100 mm	75 - 100 µL	5188-5218

Illustration of high abundance proteins removed by Agilent Multiple Affinity Removal Columns and Spin Cartridges



TIPS & TOOLS

Learn more about Agilent's complete services portfolio at **www.agilent.com/chem/services**

Multiple Affinity Removal System Starter Kits

The LC Column and Spin Cartridge Reagent Starter Kits include all the required supplies to use with Multiple Affinity Removal System. These buffers provide optimal conditions for column longevity and sample reproducibility.

- The kits provide enough Buffer A and Buffer B for approximately 200 sample depletions using the 4.6 x 50 mm LC columns, approximately 100 sample depletions using the 4.6 x 100 mm LC columns and 200 spin cartridge uses.
- Buffer A, the loading buffer, minimizes protein-protein interactions, allowing low abundant proteins often bound to high abundant proteins to pass through the column, while the targeted high abundant proteins bind to their associated antibodies.
- Buffer B, the elution buffer, then disrupts the antibody-protein interaction eluting the high abundant proteins off the column.

Multiple Affinity Removal System Starter Kits

Description	Part No.
LC Column Reagent Starter Kit Includes:	5185-5986
Buffer A, 1 L, for loading, washing, and equilibrating, qty 2	5185-5987
Buffer B, 1 L, for eluting	5185-5988
0.22 µm cellulose acetate, 25/pk, qty 2	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Multiple Affinity Removal Spin Cartridge Reagent Kit Includes:	5188-5254
Buffer A, 1 L, for loading, washing, and equilibrating	5185-5987
Buffer B, 1 L, for eluting	5185-5988
Spin filters, 0.22 µm cellulose acetate, 25/pk, qty 2	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Luer-Lok adapters, 2/pk	5188-5249
Plastic syringe, 5 mL, Luer-Lok, 2/pk	5188-5250
Microtube, 1.5 mL, screw top, 100/pk, qty 6	5188-5251
Caps and plugs, 6/pk	5188-5252
PTFE needles, Luer-Lok, 10/pk	5188-5253
High concentration sample dilution buffer, 50 mL	5188-8283

LC Column Reagent Starter Kit, 5185-5986

Luer-Lok adapters, 5188-5249

Luer-Lok syringe, 5188-5250

Luer-Lok needles, 5188-5253

mRP-C18 High-Recovery Protein Column, 4.6 x 50 mm, 5188-5231

mRP-C18 High-Recovery Protein Columns

The mRP (macroporous reversed-phase) C18 High-Recovery Protein column is designed for high recovery, high resolution separation, fractionation, and simultaneous desalting of complex protein samples (like immunodepleted serum or plasma proteins).

- Greater than 95-99% protein sample recovery has been observed with immunodepleted serum using the Agilent Multiple Affinity Removal System – LC column
- Can load up to 380 µg of total protein mass without reducing chromatographic resolution of the proteins
- Column packed with macroporous C18-bonded ultrapure 5 µm particle silica designed to reduce or eliminate strong adsorption of proteins
- Maximum operating pressure of 250 bar (4000 psi)
- Compatible with water and all common organic solvents

mRP-C18 High-Recovery Protein Columns

Description	Protein Load Capacity	Part No.
mRP-C18, 0.5 x 100 mm	10 ng - 5 µg	5188-6510
mRP-C18, 2.1 x 75 mm	8 - 85 µg	5188-6511
mRP-C18, 4.6 x 50 mm	40 - 380 µg	5188-5231

Proteomics Reagents for LC/MS Analysis

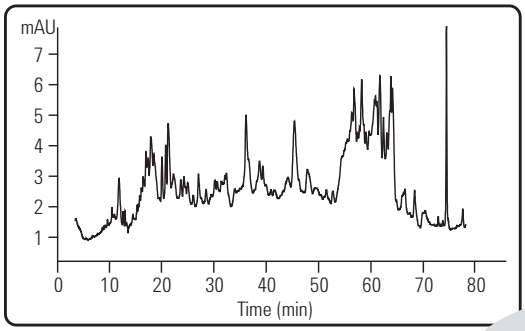
The Agilent Complex Proteomics Standard is a soluble Pfu protein extract containing over 1,500 proteins. Together with our TPCK-treated proteomics grade trypsin this is an ideal combination for workflow validation in LC/MS biomarker discovery and other proteomic studies.

Proteomics Reagents for LC/MS Analysis

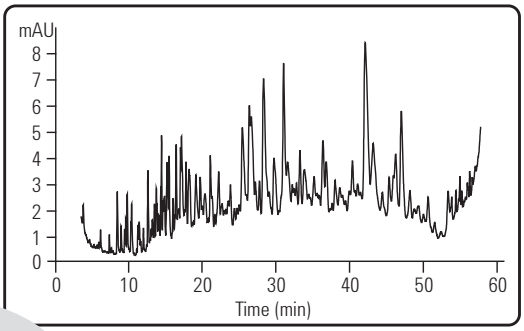
Description	Part No.
Complex Proteomics Standard	400510
Proteomics Grade Trypsin	204310

Protein Fractionation of Complex Samples on the mRP Column

mRP-C18, 4.6 x 50 mm

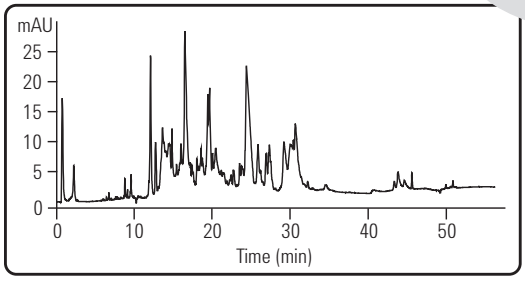


HeLa Membrane Prep

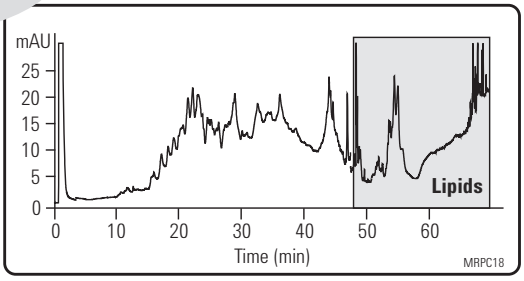


HeLa Cell Lysate (352 µg)

Highest Recovery



"Top-6" Depleted Human Serum



Human Brain Membrane Lipid Raft Prep (500 µg)

mRP-C18 High-Recovery Protein Column, 0.5 x 100 mm, 5188-6510

Method Development

ZORBAX Column Methods

This ZORBAX Column Selection Strategy for Proteins and Peptides provides some critical details on method development for proteins or polypeptides.

Choose the Initial Column and Conditions for Peptides, Polypeptides, and Proteins

Peptides, Polypeptides, Proteins MW <50 kDa	Peptides, Polypeptides, Proteins MW >50 kDa

Initial Bonded Phase

StableBond 300SB-C8

300SB columns are wide-pore columns with unbeatable lifetime in TFA-containing mobile phases. This makes them an ideal first choice for separations of peptides and proteins.

- C8 is an excellent starting bonded phase because of its moderate hydrophobicity
- C18 and C8 are generally selected for peptides and protein digests but can also be used for proteins
- C3, C4 and CN are generally selected for larger, hydrophobic polypeptides and proteins but can also be used for peptides
- PLRP-S when chemical and thermal stability are needed

Poroshell 300SB-C18

Poroshell 300SB columns use an innovative particle technology to deliver rapid protein separations. Short anaysis times with efficient peaks are easily obtained with Poroshell columns.

- C18 is a good starting bonded phase choice with Poroshell for most peptides, polypeptides and proteins because the retention is maximized
- C8 is generally selected for moderate size proteins but can be used with polypeptides or very large proteins
- C3 is generally selected for antibodies or large proteins but can be used for peptides and polypeptides

Initial Separation Conditions

Column:	**StableBond 300SB-C8** **4.6 x 150 mm, 3.5 or 5 µm** **883995-906** **863973-906**
Mobile Phase:	A: 95% H_2O: 5% ACN with 0.1% TFA B: 5% H_2O: 95% ACN with 0.085% TFA
Gradient:	0-60% B in 60 min
Temperature:	35-40 °C
Flow Rate:	1 mL/min

Column:	**Poroshell 300SB-C18** **2.1 x 75 mm, 5 µm** **660750-902**
Mobile Phase:	A: 95% H_2O: 5% ACN with 0.1% TFA B: 5% H_2O: 95% ACN with 0.085% TFA
Gradient:	0-60% B in 60 min
Temperature:	35-40 °C
Flow Rate:	2 mL/min

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. 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 completely solubilize a sample. 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 isthiocyanate
5% HOAc/6 M urea
Dilute acid + aqueous/organic solvents (ACE, MeOH, THF)
Dilute base (ammonium hydroxide)
DMSO or 0.1%-1% in DMSO
Formamide

Weakest
↓
Strongest

Increase 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:	**ZORBAX 300Extend-C18 4.6 x 150 mm, 5 µm 773995-902**	Gradient:	5-60% B in 30 min
		Temperature:	25-30 °C (<60 °C)
Mobile Phase:	A: 20 mM NH_4OH in H_2O B: 20 mM NH_4OH in 80% ACN	Flow Rate:	1 mL/min

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

Low pH		Mid and High pH
MW <50 kDa **ZORBAX 300SB-C8** **4.6 x 150 mm, 3.5 µm** 863973-906	**MW >50 kDa** **Poroshell 300SB-C18** **2.1 x 75 mm, 5 µm** 660750-902	**MW <25 kDa** **ZORBAX 300Extend-C18** **2.1 x 150 mm, 3.5 µm** 763750-902

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 also 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 id columns provide good sensitivity when sample size is not limited. With Poroshell columns, smaller 1 mm column ids are used.

Low pH		Mid and High pH
MW <50 kDa **ZORBAX 300SB-C8** **2.1 x 150 mm, 3.5 µm** 863750-906	**MW <1000 kDa** **Poroshell 300SB-C18** **1.0 x 75 mm, 5 µm** 661750-902	**MW <25 kDa** **ZORBAX 300Extend-C18** **2.1 x 150 mm, 3.5 µm** 763750-902

High Sensitivity/Proteomics Applications

Capillary columns are used for high sensitivity protein and peptide applications. The 0.5 mm id columns are used for protein and protein digest separations while the 0.3 mm id 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 id) are often used in 2-D LC/MS systems for proteomics and the initial choice is C18 bonded phase.

Bio Ion-Exchange Column Methods

Bio MAb Monoclonal Antibodies	Cation-Exchange (SCX, WCX) Monoclonal Antibodies, Proteins, Peptides, and Glycoproteins	Anion-Exchange (SAX, WAX) Oligonucleotides, Proteins

Recommended Initial Conditions

	Bio MAb Monoclonal Antibodies	Cation-Exchange (SCX, WCX) Monoclonal Antibodies, Proteins, Peptides, and Glycoproteins	Anion-Exchange (SAX, WAX) Oligonucleotides, Proteins
Mobile Phase:	A: 20 mM sodium phosphate, pH 5.5 B: Buffer A plus 400 mM NaCl	A: 20 mM sodium phosphate, pH 5.0 for WCX or pH 6.0 for SCX B: Buffer A plus 500 mM NaCl	A: 10 mM Tris buffer, pH 8 for WAX or pH 9.0 for SAX B: Buffer A plus 400 mM NaCl
Gradient:	10-35% B in 50 min	1-100% B in 30 min for 50 mm columns; 60 min for 250 mm columns	1-100% B in 30 min for 50 mm columns and 60 min for 250 mm columns
Sample Size:	2 mg/mL; 5 µL injection	2 mg/mL; 5 µL injection	2 mg/mL; 5 µL injection
Temperature:	Ambient	Ambient	Ambient
Detection:	UV 220 nm	UV 220/280 nm	UV 220/280 nm

Select Flow Rate Based on Column Diameter and Particle Size

2.1 mm id Columns		4.6 mm id Columns	
Particle Size, µm	**Flow Rate, mL/min**	**Particle Size, µm**	**Flow Rate, mL/min**
5	0.1-0.5	1.7	0.1-0.3
1.7	0.1-0.8	3	0.1-0.5
		5	0.1-0.8
		10	0.1-1.0

Optimize Conditions

Some separations may require a specific buffer, ionic strength, pH, and/or temperature

Ionic Strength:

Certain ionic strength is required to sustain the function of columns. Usually, a minimal concentration of 10-20 mM salt is required. However, greater than 20 mM strength may prevent the adsorption of biomolecules onto the column. Commonly used salts are sodium and potassium chloride and acetate. For elution, a typical salt concentration is 400-500 mM.

Note: Never use water alone for washing columns as it causes a significant increase in backpressure.

Selection of Buffers and pH:

Buffers play a key role in the optimization of separations. Phosphate buffers are typically used for antibodies and many biomolecules. The following are also recommended: MES, Tris, and ACES buffers. Use buffers of pH 5.0-6.5. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher pH (>pH 6.5) may be needed. Phosphoric acid, acetic acid, HCl and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Selection of Buffers and pH:

For anion-exchange, acetate and phosphate buffers of pH 8.0-9.0 are recommended. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher or lower pH may be needed. Phosphoric acid, acetic acid, HCl and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Additives

Organic Solvents:

Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents:

Non-ionic, anionic, and zwitterionic detergents can be used. Cationic detergents are not recommended.

Additives

Organic Solvents:

Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents:

Non-ionic, cationic, and zwitterionic detergents can be used. Anionic detergents are not recommended.

Temperature:

Agilent Bio MAb and IEX columns are stable up to 80 °C. However, many proteins and biomolecules are heat labile. Be sure to establish the temperature stability of your sample before routinely using high temperature for separation.

SEC Column Methods

Choose Initial Columns and Conditions for Size-Based Separation of Biomolecules, Aggregation Analysis – Peptides, Polypeptides, and Proteins

Peptides, Polypeptides, Proteins MW >0.1-1,250 kDa

Peptides, Polypeptides, Proteins MW >0.1-10,000 kDa

Select Column Based on Molecular Weight Range and Pore Size

Agilent Bio SEC-3 (3 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250

Agilent Bio SEC-5 (5 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250
500Å	15-5,000
1000Å	50-7,500
2000Å	>10,000

Recommended Initial Separation Conditions

Column: Agilent Bio SEC (3 µm and 5 µm)

Mobile Phase: 150 mM phosphate buffer, pH 7.0*

Gradient: Isocratic in 30-60 min range

Temperature: Recommended: 10-30 °C, Maximum: 80 °C

Flow Rate: 0.1-0.4 mL/min for 4.6 mm id columns
0.1-1.25 mL/min for 7.8 mm id columns

Sample Size: ≤ 5% of total column volume

*Other aqueous buffers with high and low salt can be used

For additional information, see application note: *Defining the Optimum Parameters for Efficient Size Separations of Proteins* (publication # 5990-8895EN)
www.agilent.com/chem/library

After the initial chromatogram, additional changes may be needed to improve the separation, maintain protein solubility, or to decrease sample interaction with the chromatographic media. The ionic strength of the mobile phase can be adjusted up or down in strength to attain an optimized separation. pH can also be adjusted usually + 0.2 units. If further optimization is necessary, the upward or downward range should be expanded. A change of temperature or addition of an organic solvent can also be used.

For protocols requiring additional salt, these buffers are typical:

100-150 mM sodium chloride in 50 mM sodium phosphate, pH 7.0

100-150 mM sodium sulfate in 50 mM sodium phosphate, pH 7.0

50-100 mM urea in 50 mM sodium phosphate, pH 7.0

Other similar salts (e.g. KCl) and guanidine hydrochloride can also be used

pH Range:

2.0-8.5

Potential organic solvent additions include:

5-10% ethanol (or other similar solvents) in 50 mM sodium phosphate, pH 7.0

5% DMSO in 50 mM sodium phosphate, pH 7.0

Temperature:

Typically, SEC separations are run at 20-30 °C. Separation of proteins and peptides may require higher temperature to improve both resolution and recovery of proteins and hydrophobic peptides.

Maximum temperature of Bio SEC columns is 80 °C

High Sensitivity Capillary Column Methods

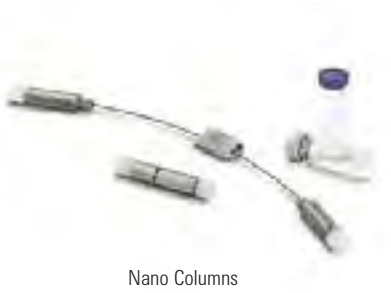
Mobile Phase Considerations

Low pH

TFA is generally not used for LC/MS separations of proteins and peptides. The first step is normally to replace TFA with 0.1 to 1% formic acid. Acetic acid, up to 1% can also be used as an alternative mobile phase modifier. At low pH, the best separation may still be obtained with TFA in the mobile phase. In some cases, the TFA can be displaced post-column with an alternative acid, such as propionic acid.

Mid and High pH

LC/MS can also be done at high pH with 10-20 mM NH_4OH as a mobile phase additive.



Nano Columns

Capillary and Nano Columns

- 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 and 0.3 mm id) and Nano (0.1 and 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.

TIPS & TOOLS

Agilent offers a variety of e-Seminars and on-site training to help you learn how to be a more effective chromatographer.

For more information, visit
www.agilent.com/chem/education

ZORBAX Nano columns for high sensitivity protein digest analysis by LC/MS

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

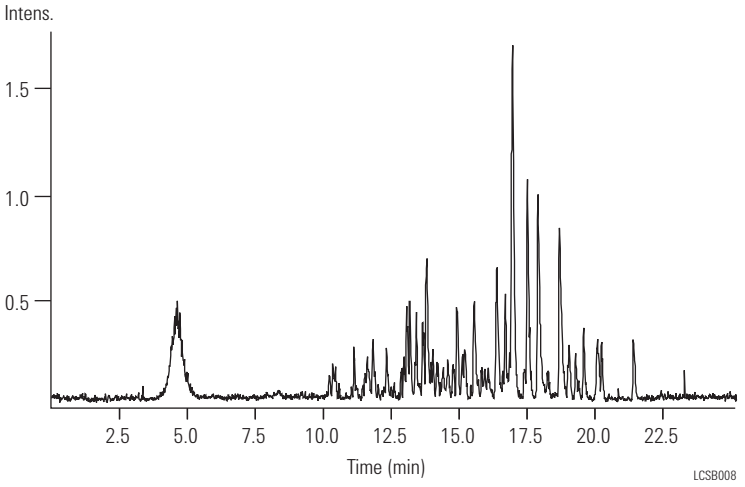
Mobile Phase: A: Water + 0.1% Formic acid,
B: ACN + 0.1% Formic acid

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.

High sensitivity with capillary columns

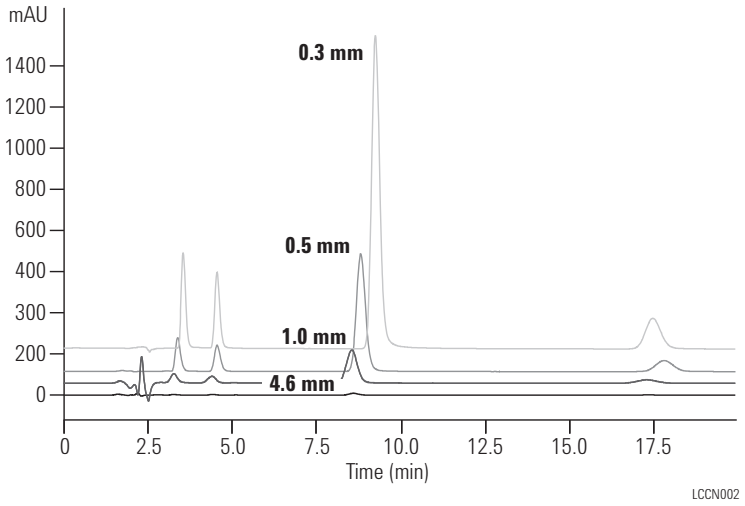
Column: **ZORBAX SB-C18**
5064-8255
0.3 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
5064-8256
0.5 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
863600-902
1.0 x 150 mm, 3.5 µm

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

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. Agilent Nanobore (0.1 mm to 0.075 mm id) columns can provide up to 2,000 times more sensitivity for your most limited sample applications.

Human serum: Low abundance protein isolation and identification from 1-D gel band by LC/MS

Column: **ZORBAX 300SB-C18**
Trap: 0.3 x 5 mm, 5 µm, 5065-9913
Analytical: 0.3 x 150 mm, 5 µm, 5064-8263

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 6 µL/min

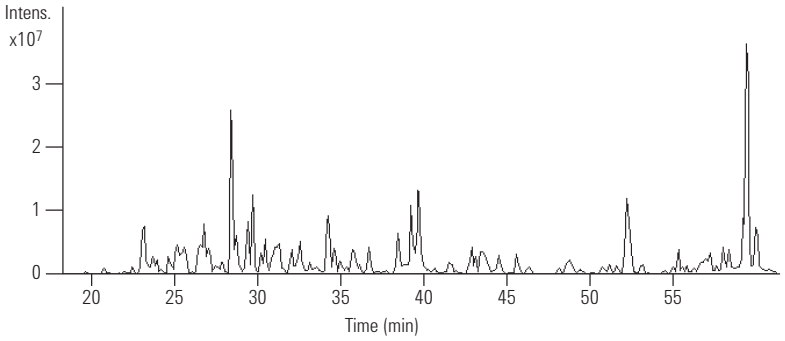
Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

Sample: Band from 1-D in gel digest

Proteins Identified

1. α-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

Base Peak Chromatogram



Sample Preparation of Human Serum:
Major serum proteins removed using Multiple Affinity Removal Column:
4.6 x 100 mm, P/N 5185-5985
Followed by 1-D gel digest

Peptide phosphorylation sites LC and LC/MS using Capillary LC columns

Column: **ZORBAX 300SB-C18**
5064-8268
0.5 x 150 mm, 3.5 µm

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 5.5 µL/min

Gradient: 5-55% B in 50 min, to
85% B from 55-57 min

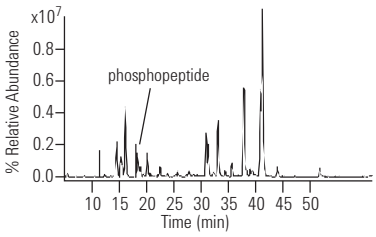
Detector: UV, 206 nm

MS Conditions:

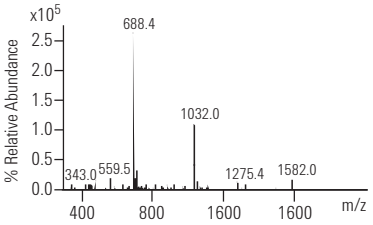
LC/MS:	Pos. Ion ESI with LC/MSD trap
Vcap:	4000 V
Drying gas flow:	7 L/min
Drying gas temperature:	250 °C
Nebulizer:	15 psi
Capillary Exit Volt:	50 V Max
Accum Time:	300 ms
Total Averages:	3
Isolation Width:	3 m/z
Frag Ampitude:	1.0 V

Sample: Beta case in digest, 100 nL (4 pmol)

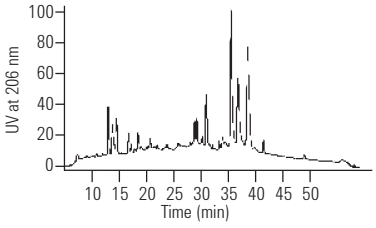
MS



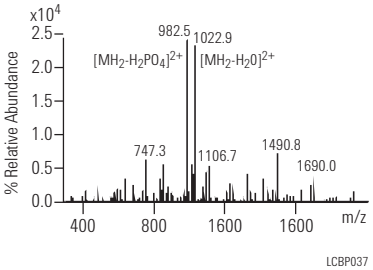
Full Scan MS



UV



MS/MS of [M+2H]$^{2+}$ at m/z 1032



Capillary columns for HPLC analyses with UV and MS detection

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

Mobile Phase: 5-55% B in 50 min, to 85% B from 55-57 min
A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

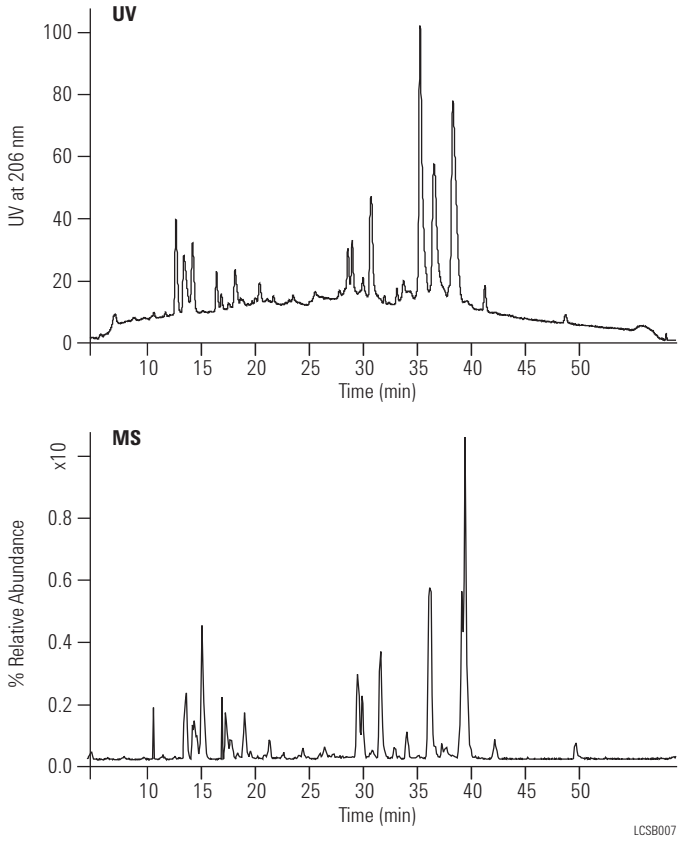
Flow Rate: 5.5 µL/min

Detector: UV, 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 Ampitude: 1.0 V

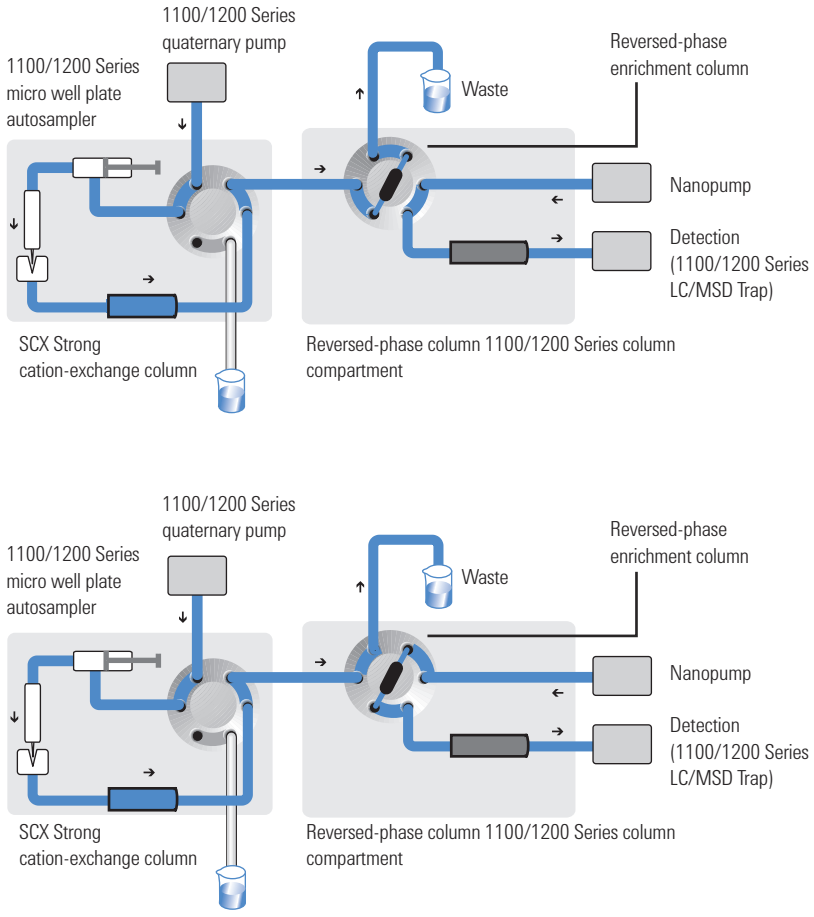
Sample: 100 nL
Beta Casein Digest (4 pmol)

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.



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

Typical Column Configuration for 2-D HPLC



Flow path of an Agilent customized Nanoflow Proteomics 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 anaylsis

Proteins in a complex sample by 2-D HPLC with Nano HPLC columns

Column: **ZORBAX 300SB-C18**
5065-9913
0.3 x 5 mm, 5 µm

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

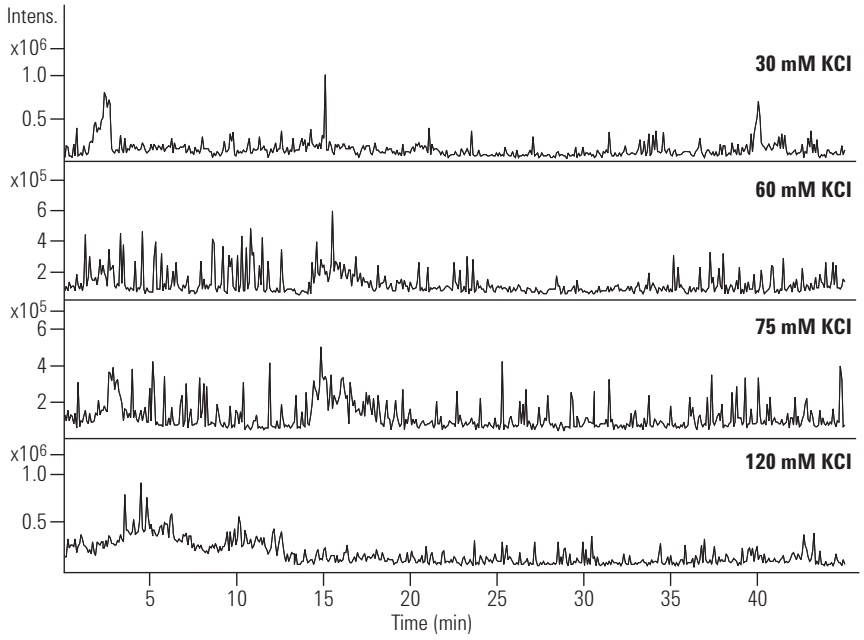
Mobile Phase: Quaternary Pump: 3% Acetonitrile/0.1% Formic acid
Nanopump: A = Water, 0.1% Formic acid, B = ACN, 0.1% Formic acid

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: 5 L/min, drying gas temp: 225 °C
Ion Trap: Skim: 1:35 V, cap exit offset: 115 V, octupole 1:12 V, octupole 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: 8 mL of 10 mM-100 mM KCl (10 mM increments), 125 mM, 150 mM, 200 mM, 300 mM, 500 mM, 1 M.



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

Nano Columns

ZORBAX Bio-SCX Series II

ZORBAX 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 Bio-SCX Series II	300Å	90 m^2/g	2.5-8.5	Sulfonic acid	350 bar

ZORBAX Bio-SCX Series II

Description	Size (mm)	Particle Size (µm)	Bio-SCX Series II
Capillary	0.3 x 35	3.5	5065-9912
Capillary	0.8 x 50	3.5	5065-9942

ZORBAX Bio-SCX Series II provides more retention of small peptides

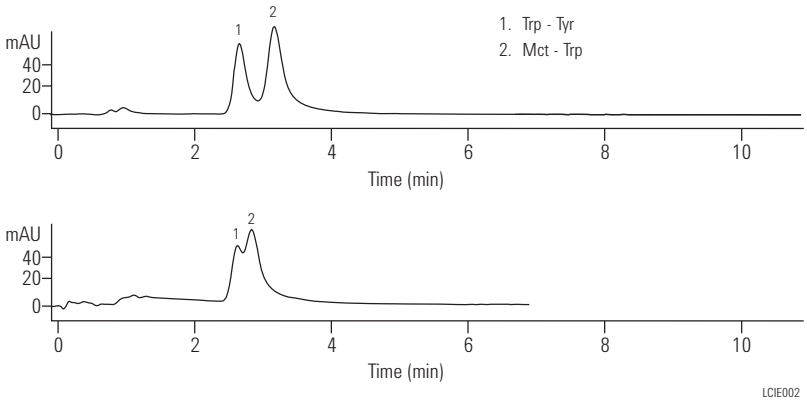
Column: **ZORBAX Bio SCX Series II 5065-9912 0.3 x 35 mm, 3.5 µm**

Mobile Phase: 95% 40 mM NaCl: 5% ACN, 0.3% Formic acid

Flow Rate: 5 µL/min

Detector: 230 nm

Sample: Synthetic Dipeptides



LCIE002

The new ZORBAX Bio-SCX Series II column retains smaller peptides more strongly than some other SCX columns. The result is increased resolution of more hydrophilic peptides fragments and more accurate identification when these columns are used in 2-D HPLC analysis.

ZORBAX HPLC Capillary Columns (glass-lined stainless steel)

Description	Size (mm)	Particle Size (µm)	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	5064-8266				
Capillary	0.5 x 150	5	5064-8264				
Capillary RR	0.5 x 150	3.5	5064-8268				
Capillary	0.5 x 75	5			5065-4468		
Capillary	0.5 x 35	5	5064-8294				
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	0.3 x 35	3.5					5065-9912
Capillary RR	0.3 x 150	3.5	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	

ZORBAX 300SB-C18 trap/guard, 5065-9913

ZORBAX Nano HPLC Columns (PEEK)

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

MicroBore (1.0 mm id) Columns

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

Agilent MicroBore (1.0 mm id) columns are 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 µL/min). Therefore, these columns are ideal for use with detectors requiring low flow rates such as some mass spectrometers and with capillary LC systems.

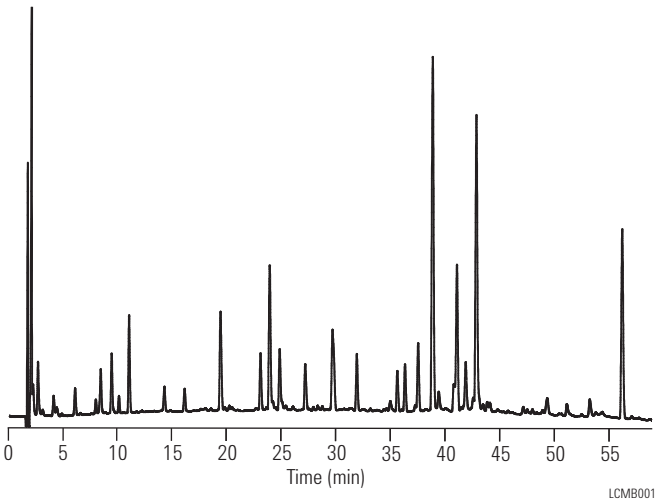
Optimum performance is achieved when MicroBore columns are used with UHPLC/HPLC Microbore systems. A wide variety of bonded phases is available for use up to 400 bar including StableBond, 300SB-C18, 300SB-C8, and Poroshell columns. Polymeric reversed-phase, PLRP-S, and ion-exchange PL-SAX and PL-SCX are also available for applications requiring exceptionally stable wide pore particles. Guard columns are also now available with an adjustable tube stop depth to provide a perfect zero dead volume connection every time.

Sterically Protected 300StableBond Bonded Phase

Separation of a tryptic digest on ZORBAX MicroBore 300SB-C18

Column:	**ZORBAX 300SB-C18** **863630-902** **1.0 x 150 mm, 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 µL/min
Temperature:	50 °C
Detector:	UV, 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.



Microbore HPLC for sensitive peptide analysis

Column: **PLRP-S 100Å 5 µm, 150 mm x various id**

Mobile Phase: A: 0.01 M Tris HCl, pH 8
B: A + 0.35 M NaCl, pH 8

Flow Rate: 1 mL/min

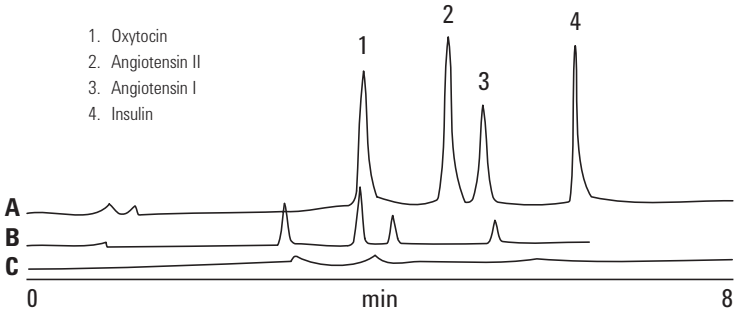
Gradient: Linear 20% ACN, 0.1% TFA to 50% ACN, 0.1% TFA over 15 min

Injection Volume: 0.5 µL

Sample Conc: 0.25 mg/mL

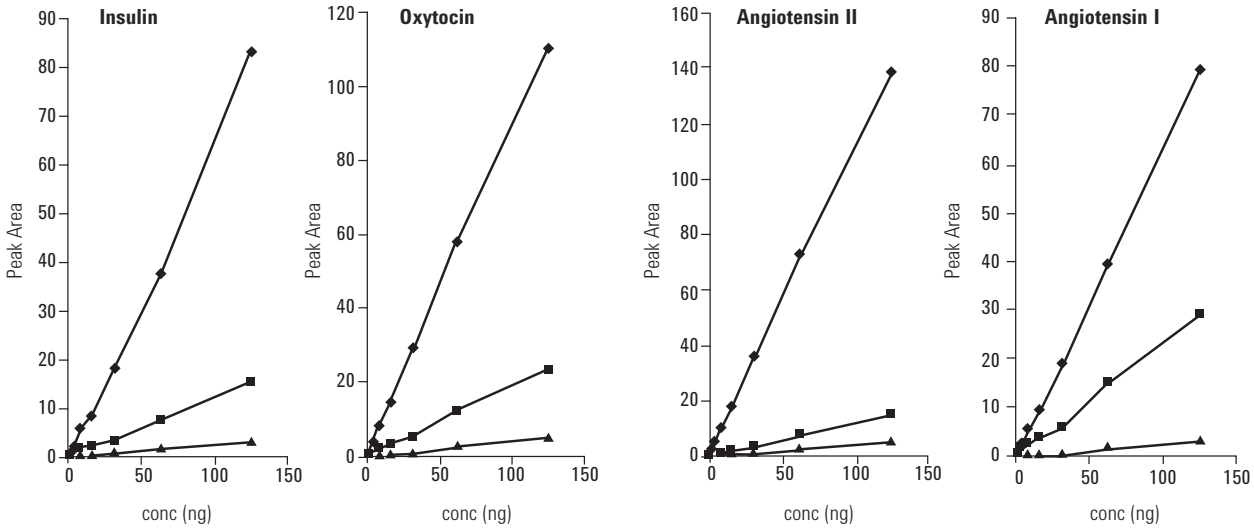
Detector: UV, 220 nm

Peak Identification
A. 1.0 mm id (flow rate 47 µL/min)
B. 2.1 mm id (flow rate 200 µL/min)
C. 4.6 mm id (flow rate 1 mL/min)



Peptide separation on Agilent PLRP-S 100Å 5 µm columns

Peak Identification
◆ 1.0 mm
■ 2.1 mm
▲ 4.6 mm



Standard curve data-point graphs on Agilent PLRP-S columns

MicroBore (1.0 mm id)

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7		
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, 3/pk	1.0 x 17	5	5185-5920	5185-5920		

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, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	

Description	Size (mm)	Particle Size (µm)	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
MicroBore	1.0 x 150	3	PL1312-3300			
MicroBore	1.0 x 50	3	PL1312-1300	PL1312-1301		
MicroBore	1.0 x 50	5	PL1312-1500	PL1312-1501	PL1312-1502	PL1312-1503
MicroBore	1.0 x 50	8			PL1312-1802	PL1312-1803

Description	Size (mm)	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
MicroBore	1.0 x 50	5	PL1351-1502	PL1351-1503	PL1345-1502	PL1345-1503

Polymeric Prep HPLC Columns

Purification – Prep HPLC

Agilent has a comprehensive range of silica and polymeric HPLC columns and media designed for biomolecule purification. There are high efficiency small particle prep columns optimized for the purification of µg and mg amounts of a biopharmaceutical drug candidate and fully porous bulk media, to pack development and process columns to purify multiple 100 g, kg and multi-kg of API.

Some columns are specifically designed to address the needs of high efficiency purification, while other products provide easy scale-up from small particle analytical columns to full scale API production. **Table 1** shows prep column/media options and the quantity of product that can be purified.

BioPharmaceutical Lifecycle		Discovery	Development	Production
		µg mg	g	kg multi-kg
		high efficiency		high throughput
Reversed-Phase	**mRP-C18**	→		
	ZORBAX Prep HT 300Å StableBond	→	→	
	VariTide RPC	→	→	
	PLRP-S 100Å, 300Å, 1000Å, 4000Å	→	→	→
	PL-SAX	→	→	→
Ion-Exchange	**PL-SCX**	→	→	→
Size Exclusion	**ZORBAX GF-250/450**	→	→	

Table 1: Agilent columns and media for biomolecule purification – chromatographic type, product family and purification scale.

Purification Column Selection

Application	Technique	Notes	Agilent Columns
Proteomics	Reversed-Phase	A specialist high recovery column for proteomics applications. It is designed for µg scale purifications with maximum recovery.	mRP-C18
All Biomolecules	Reversed-Phase	High efficiency 300Å silica-based particles.	ZORBAX PrepHT 300SB
Synthetic Peptides	Reversed-Phase	Polymeric material designed for the purification of synthetic peptides. It is a high efficiency single-column solution for the full range of synthetic peptides, acidic, basic, hydrophobic and hydrophilic, and covers the size range of peptides produced by both solution and solid phase synthesis.	VariTide RPC
All Biomolecules	Reversed-Phase	The premium polymeric reversed-phase family with a range of pore sizes and particle sizes to enable high efficiency laboratory scale purification using small particle prep column, and scale-up to high yield production purification with larger particles at the process scale. Use PLRP-S when purification will be scaled up to produce APIs and will need regulatory documentation.	PLRP-S
		• 3 µm and 5 µm for high efficiency • 8 µm, 10 µm, 10-15 µm, 15-20 µm, 30 µm and 50 µm particles for larger scale and low pressure purification	
All Biomolecules	Ion-Exchange	A fully porous strong anion-exchanger	PL-SAX
		• 5 µm particle size for high efficiency separations • 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification	
		A fully porous strong cation-exchanger	PL-SCX
		• 5 µm particle size for high efficiency separations • 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification	

TIPS & TOOLS

Further information can be found in the following publication:

Biomolecule Purification
(publication # 5990-8335EN)

www.agilent.com/chem/library

ZORBAX 300Å StableBond Prep HT Cartridge Columns

ZORBAX PrepHT

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 – StableBond 300Å, C18, C8, C3, and CN – 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.

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
PrepHT Cartridge Columns (require endfittings kit 820400-901)							
	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109
	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109
	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909
	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909
	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901
	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924
	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901

PLRP-S for Prep to Process

- Discovery stage to multi-kg cGMP production reduces method development time
- Chemical stability for separations, optimization, sanitation, and regeneration increases selectivity and column lifetime
- Single batch packing of multiple columns reduces system downtime and validation costs

The PLRP-S media, rigid poly(styrene/divinylbenzene) particles, are available in a range of pore sizes for small molecule, synthetic biomolecule and macromolecule purification. Their thermal and chemical stability makes them ideal for purifications that require extreme conditions for sample preparation, compound elution, and column regeneration.

Capacity and resolution are two key parameters for maximizing the throughput of a purification. With a wide choice of pore sizes and extended range of operating conditions, PLRP-S provides more options to achieve the optimum process. Particle sizes range from 3 µm to 50 µm for scale-up from the µg/mg discovery stage to multi-kg cGMP production. Excellent chemical stability, up to 1 M NaOH, permits sanitation and regeneration that increase column lifetime. PLRP-S media batch sizes of up to 600 L are available, providing single batch packing of multiple columns.

As part of our commitment to quality and continuity of supply, all manufacturing is carried out under a fully documented process. A Type II Drug Master File and regulatory support files are available for process materials, and facility audits are routinely conducted.

PLRP-S Prep to Process Application Guide

Application	PLRP-S Media Pore Size			
	100Å	300Å	1000Å	4000Å
Synthetic biomolecules, peptides, and oligonucleotides	✓	✓		
Recombinant biomolecules, peptides, and proteins	✓	✓		
Large biomolecules, antibodies, DNA fragments			✓	✓
Small molecules, unstable compounds including metal sensitivity	✓			

Column Specifications

pH Range	1-14
Buffer Content	Unlimited
Organic Modifier	1-100%
Temperature Limits	200 °C
Maximum Pressure	5-8 µm: 3000 psi (210 bar)
	3 µm: 4000 psi (300 bar)

Purification of a 25-mer trityl-off oligonucleotide and analytical quantitation of the fraction using PLRP-S 100Å, 4.6 x 50 mm

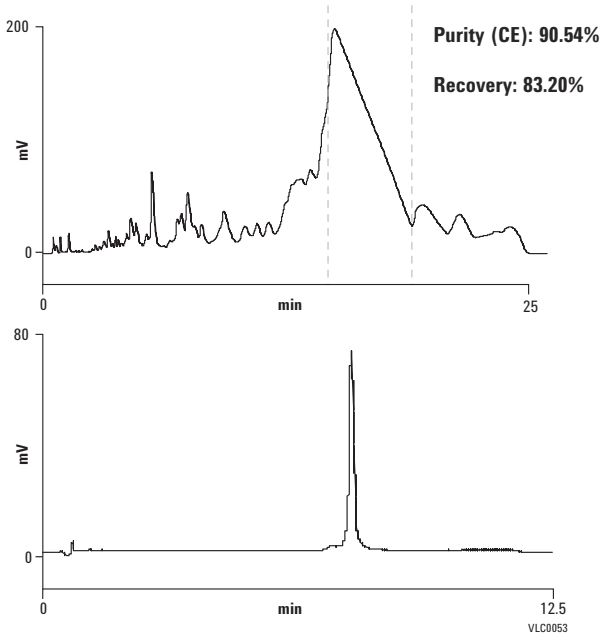
Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

Mobile Phase: A: 100 mM Triethylammonium acetate (TEAA)
B: 100 mM TEAA in 25:75 Acetonitrile:water

Flow Rate: 1 mL/min

Gradient: 25% B 0 min, 35% B 2 min, 45% B 22.5 min, 45% B 23 min, 25% B 23.05 min, 25% B 26 min

Temperature: 80 °C



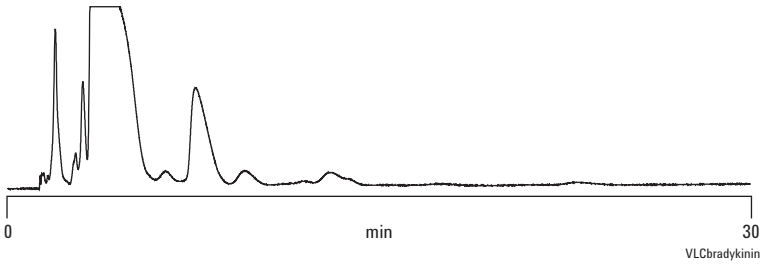
Crude bradykinin prep load

Column: **PLRP-S 100Å**
PL1512-5100
4.6 x 250 mm, 10 µm

Sample: 30 µL containing 1.5 mg of crude peptide

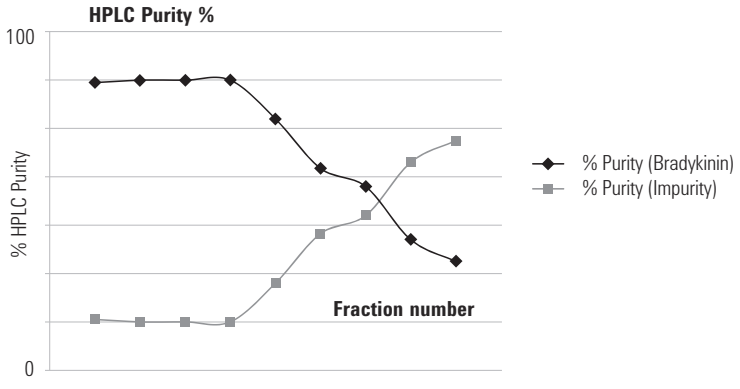
Mobile Phase: 0.1% TFA in 21% ACN:79% water

Flow Rate: 1 mL/min (360 cm/hr)



Fraction analysis – the concentration overload purification

HPLC analysis of the fractions collected across the peak showed that fractions 1 to 4 contained only the peptide of interest and that the level of the critical impurity increased with increasing fraction number. Using the high efficiency PLRP-S column it was possible to obtain from the crude, 91.7% pure, a recovery of 97% with 100% purity. For more information, see application note 5990-7736EN.



Prep to Process PLRP-S

Size (mm)	Particle Size (µm)	PLRP-S 100Å	PLRP-S 300Å	PLRP-S 1000Å	PLRP-S 4000Å
100 x 300	30			PL1812-3102	PL1812-3103
100 x 300	15-20	PL1812-6200	PL1812-6201		
100 x 300	10-15	PL1812-6400	PL1812-6401		
100 x 300	10	PL1812-6100	PL1812-6101		
100 x 300	8	PL1812-6800	PL1812-6801		
50 x 300	8	PL1712-6800	PL1712-6801		
50 x 150	30			PL1712-3702	PL1712-3703
50 x 150	15-20	PL1712-3200	PL1712-3201		
50 x 150	10-15	PL1712-3400	PL1712-3401		
50 x 150	10	PL1712-3100	PL1712-3101	PL1712-3102	PL1712-3103
50 x 150	8	PL1712-3800	PL1712-3801		
25 x 300	15-20	PL1212-6200	PL1212-6201		
25 x 300	10-15	PL1212-6400	PL1212-6401		
25 x 300	10	PL1212-6100	PL1212-6101		
25 x 300	8	PL1212-6800	PL1212-6801		
25 x 150	30			PL1212-3702	PL1212-3703
25 x 150	10	PL1212-3100	PL1212-3101	PL1712-3102	PL1712-3103
25 x 150	8	PL1212-3800	PL1212-3801		
25 x 50	10			PL1212-1102	PL1212-1103
PLRP-S Method Development Columns					
4.6 x 250	30			PL1512-5702	PL1512-5703
4.6 x 250	15-20	PL1512-5200	PL1512-5201		
4.6 x 250	10-15	PL1512-5400	PL1512-5401		
4.6 x 250	10	PL1512-5100	PL1512-5101	PL1512-5102	PL1512-5103
4.6 x 250	8	PL1512-5800	PL1512-5801		
4.6 x 150	30			PL1512-3702	PL1512-3703
4.6 x 150	15-20	PL1512-3200	PL1512-3201		
4.6 x 150	10-15		PL1512-3401		
4.6 x 150	10	PL1512-3100	PL1512-3101	PL1512-3102	PL1512-3103
4.6 x 150	8	PL1512-3800	PL1512-3801		

PLRP-S Bulk Media

Particle Size (µm)	Unit	PLRP-S 100Å	PLRP-S 300Å	PLRP-S 1000Å	PLRP-S 4000Å
50	1 kg	PL1412-6K00	PL1412-6K01	PL1412-6K02	
	100 g	PL1412-4K00	PL1412-4K01	PL1412-4K02	
30	1 kg			PL1412-6702	PL1412-6703
	100 g			PL1412-4702	PL1412-4703
15-20	1 kg	PL1412-6200	PL1412-6201		
	100 g	PL1412-4200	PL1412-4201		
10-15	1 kg	PL1412-6400	PL1412-6401		
	100 g	PL1412-4400	PL1412-4401		
10	1 kg	PL1412-6100	PL1412-6101	PL1412-6102	PL1412-6103
	100 g	PL1412-4100	PL1412-4101	PL1412-4102	PL1412-4103
8	1 kg	PL1412-6800	PL1412-6801		

For larger quantities, please contact your local Agilent sales office

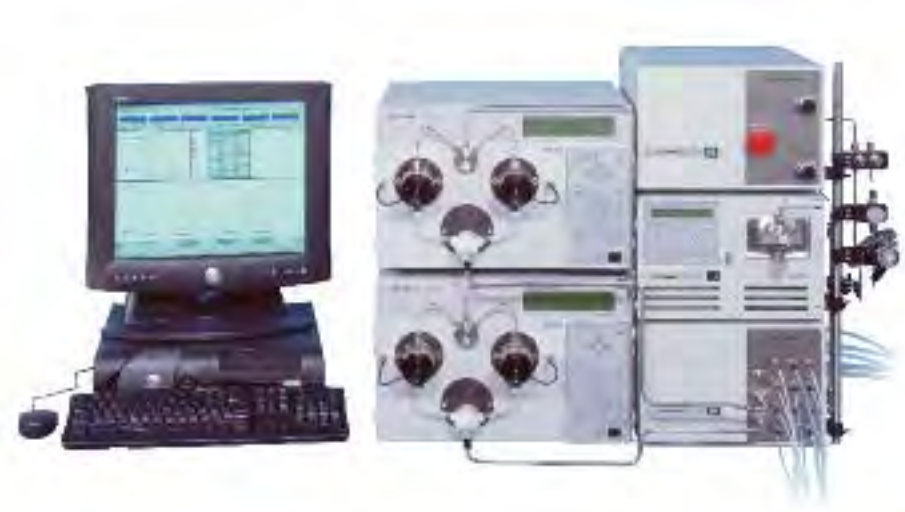
PL-SAX and PL-SCX for Prep to Process

- Ion-exchange purifications over a wider pH range extend applications
- HPLC flow rates and rapid equilibration reduce purification cycle times
- Large pore size for improved mass transfer delivers high speed, high resolution purifications

These rigid, strong ion-exchange materials are extremely hydrophilic and are designed for purification of biomolecules. The PL-SAX and PL-SCX materials are totally polymeric and are chemically and thermally stable over a full range of HPLC conditions. The strong ion-exchange functionalities, covalently linked to a chemically stable polymer, facilitate ion-exchange purifications over a wider pH range. This stability can be exploited for column sanitation and clean-up. Thermal stability also enables the use of denaturing conditions and stabilizing/solubilizing agents for the purification of target compounds, as encountered in the purification of synthetic oligonucleotides with self-complementary sequences.

Both the 1000Å and 4000Å wide-pore materials are mechanically stable and robust and can be operated over a wide range of linear velocities, with fast loading of dilute solutions and wash cycles. HPLC flow rates and rapid equilibration reduces purification cycle times.

Packing in dynamic axial compression (DAC) column hardware is straightforward and high efficiency columns are achieved with excellent reproducibility and lifetimes. The 1000Å pore size is for high-capacity purifications and the 4000Å gigaporous particles with improved mass transfer are intended for large biomolecules and high-speed, high-resolution purifications.



Column Specifications		
	PL-SAX	**PL-SCX**
Matrix	Fully polymeric	Fully polymeric
Pore Sizes	1000Å, 4000Å	1000Å, 4000Å
Particle Sizes	10 µm, 30 µm	10 µm, 30 µm
Bead Form	Rigid spherical	Rigid spherical
Functionality	Quaternary amine	Sulfonic acid
Pressure Stability	3000 psi	3000 psi
Temperature Stability	80 °C	80 °C
pH Range	1-14	1-14
Eluent Compatibility	All anion-exchange buffers	All cation-exchange buffers
Packed Bed Density	0.39 g/mL	0.39 g/mL

Purification of a large oligonucleotide

Column: **PL-SAX 1000Å, 8 µm**

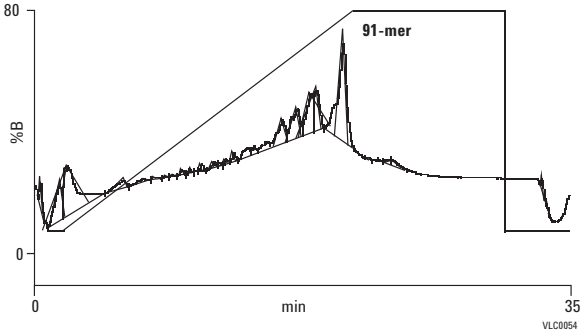
Mobile Phase: A: 93% 0.1 M TEAA, pH 7:7% ACN
B: 93% 0.1 M TEAA, 3.24 M ammonium acetate, pH 7:7% ACN

Gradient: 0-100% B in 20 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 290 nm



Preparative fractionation of a culture filtrate containing amyloglucosidases on Agilent PL-SAX 4000Å

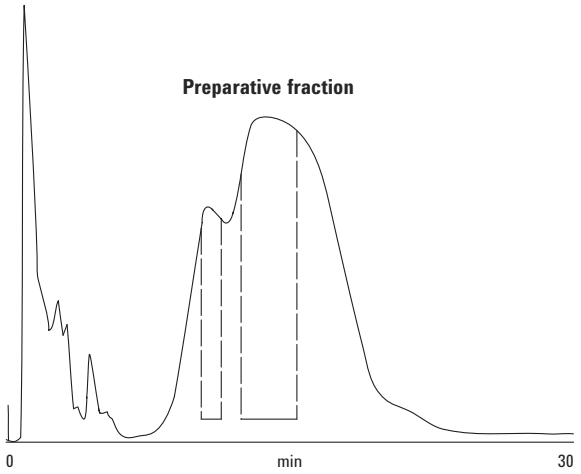
Column: **PL-SAX**
PL1551-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.01 M Tris HCl, pH 8
B: A + 0.5 M NaCl, pH 8

Flow Rate: 4.0 mL/min

Gradient: Linear 0-100% B in 2 min

Detector: UV, 280 nm



Prep to Process PL-SAX and PL-SCX Columns and Bulk Media

Prep to Process PL-SAX and PL-SCX

Dimensions	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
100 x 300	30	PL1851-3102	PL1851-3103	PL1845-3102	PL1845-3103
100 x 300	10	PL1851-2102	PL1851-2103	PL1845-2102	PL1845-2103
50 x 150	30	PL1751-3702	PL1751-3703	PL1745-3702	PL1745-3703
50 x 150	10	PL1751-3102	PL1751-3103	PL1745-3102	PL1745-3103
25 x 150	30	PL1251-3702	PL1251-3703	PL1245-3702	PL1245-3703
25 x 150	10	PL1251-3102	PL1251-3103	PL1245-3102	PL1245-3103
25 x 50	10	PL1251-1102	PL1251-1103	PL1245-1102	PL1245-1103
7.5 x 150	8	PL1151-3802	PL1151-3803		
7.5 x 50	8	PL1151-1802	PL1151-1803	PL1145-1802	PL1145-1803
PL-SAX and PL-SCX Method Development Columns					
4.6 x 250	30	PL1551-5702	PL1551-5703	PL1545-5702	PL1545-5703
4.6 x 250	10	PL1551-5102	PL1551-5103	PL1545-5102	PL1545-5103
4.6 x 150	30	PL1551-3702	PL1551-3703	PL1545-3702	PL1545-3703
4.6 x 150	10	PL1551-3102	PL1551-3103	PL1545-3102	PL1545-3103

PL-SAX and PL-SCX Bulk Media

Particle Size (µm)	Unit	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
30	1 kg	PL1451-6702	PL1451-6703	PL1445-6702	PL1445-6703
	100 g	PL1451-4702	PL1451-4703	PL1445-4702	PL1445-4703
10	1 kg	PL1451-6102	PL1451-6103	PL1445-6102	PL1445-6103
	100 g	PL1451-4102	PL1451-4103	PL1445-4102	PL1445-4103

For larger quantities, please contact your local Agilent sales office

Peptide Purification

VariTide RPC Columns

VariTide is a cost-effective solution for the production of synthetic peptides. This column lets you manage the cost and efficiency of high-volume synthetic peptide purification, from µg to g scale. VariTide provides a solution for peptide houses that manufacture small quantities of hundreds or thousands of peptides where manufacturing time is the economic driving force.

VariTide RPC Columns for Synthetic Peptides

- A single column to cover the full range of synthetic peptides
- Small particle size for maximum efficiency, even with 1 and 2 in prep columns
- Bulk media to pack 1 and 2 in prep columns for the purification of mg to g quantities

VariTide RPC columns and media are part of the VariPep Peptide Solution. This is the recommended option for cost-effective separation and purification of synthetic peptides using generic methods.

VariTide RPC Columns for Synthetic Peptides

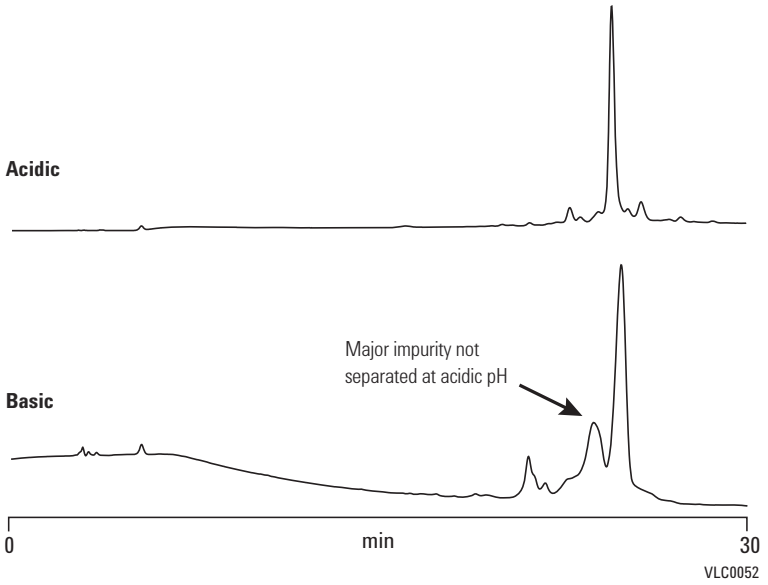
Size (mm)	Part No.
21.2 x 250	PL1E12-5A05
10.0 x 250	PL1012-5A05
4.6 x 250	PL1512-5A05

VariTide RPC Bulk Media

Description	Part No.
100 g	PL1412-4A05
1 kg	PL1412-6A05

Crude peptide screen

Column:	**VariTide RPC PL1512-5A05 4.6 x 250**
Mobile Phase:	Acidic A: 0.1% TFA in 95% water: 5% ACN B: 0.1% TFA in 50% water: 50% ACN Basic A: 5% ACN, 95% 20 mm ammonium carbonate pH 9.5 B: 50% ACN, 50% 20 mm ammonium carbonate pH 9.5
Flow Rate:	1.0 mL/min (360 cm/h)
Gradient:	0-100% B in 30 min
Detector:	UV, 220 nm



VariPure IPE

- Pre-packed for convenience
- Removal of ion-pairing agents for improved productivity
- High performance and economy for excellent efficiency

VariPure IPE is a polymer-supported quaternary-amine resin with a bicarbonate counter ion, designed for removing acidic ion-pair reagents, such as trifluoroacetic acid (TFA), formic acid or acetic acid. VariPure IPE is a high performance and economical acid removal material conveniently supplied as pre-packed SPE type devices. The particle size, capacity and device geometry are matched to provide sufficient residence time to achieve effective ion-air extraction under gravity flow. For acid labile peptides, removal of the ion-pairing agent prevents acid degradation of the peptide during post-HPLC work-up, and increases the yield of purified product.

VariPure IPE

Loading	Counter-ion Removal Capacity	Unit	Part No.
100 mg per 3 mL tube	~ 5 mL 0.1% TFA	50/pk	PL3540-D603VP
500 mg per 6 mL tube	~ 25 mL 0.1% TFA	50/pk	PL3540-C603VP
1 g per 20 mL tube	~ 50 mL 0.1% TFA	25/pk	PL3540-P603VP
25 g			PL3549-3603VP

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Rapid Analysis of Adenovirus Type 5 Particles with Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform	Bio-Monolith QA	Adenovirus	5990-5524EN	Application Note
Separation of Two Sulfurated Amino Acids with other Seventeen Amino Acids by HPLC with Pre-Column Derivatization	Eclipse Plus-C18	Amino acid analysis	5990-5977EN	Application Note
Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids	ZORBAX Eclipse AAA	Amino acid analysis	5980-1193EN	Application Note
High-Speed Amino Acid Analysis (AAA) on 1.8 µm Reversed-Phase (RP) Columns	ZORBAX Eclipse Plus	Amino acid analysis	5989-6297EN	Application Note
Improved Amino Acid Methods Using Agilent ZORBAX Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals	ZORBAX Eclipse Plus	Amino acid analysis	5990-4547EN	Application Note
Rapid and Precise Determination of Cellular Amino Acid Flux Rates using HPLC with Automated Derivatization with Absorbance Detection	ZORBAX Eclipse Plus	Amino acid analysis	5990-3283EN	Application Note
Agilent PL-SAX 1000Å HPLC Columns and Media	PL-SAX	Analysis/Prep - Oligonucleotides	5990-8200EN	Flyer
Compliance for Biopharmaceutical Laboratories	LC columns	Compliance	5990-7001EN	Primer
Macroporous Reversed-Phase C18 High-Recovery Protein Fractionation HPLC Column	mRP-C18	Human serum, Biomarkers	5989-2714EN	Brochure
Rapid Human Polyclonal IgG Quantification using the Agilent Bio-Monolith Protein A HPLC Column	Bio-Monolith	IgG	5989-9733EN	Application Note
Rapid IgM Quantification in Cell Culture Production and Purification Process Monitoring using the Agilent Bio-Monolith QA Column	Bio-Monolith QA	IgM	5989-9674EN	Application Note
Optimization of Protein Separations on Weak Cation-Exchange Columns – a Study of the Particle Size, Buffer Salts and Gradients	Bio IEX	MAbs	5990-8833EN	Technical Poster

(Continued)

BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
pH Gradient Elution for Improved Separation of Monoclonal Antibody Charged Variants	Bio MAb	MAbs	5990-9629EN	Application Note
Characterization of Monoclonal Antibodies on the Agilent 1260 Infinity Bio-inert Quaternary LC by Size Exclusion Chromatography using the Agilent Bio SEC Columns	Bio SEC	MAbs	5990-6414EN	Application Note
Agilent BioHPLC Columns for the Characterization of Monoclonal Antibodies	Biocolumns	MAbs	5990-7753EN	Flyer
Fast Separation of Monoclonal Antibody and Dimer by SEC with Agilent Bio SEC	Bio SEC	MAbs	5990-8613EN	Application Note
Choosing a ZORBAX Poroshell Phase (C3, C8, or C18) for Fast Separation of Monoclonal Antibodies	Poroshell 300	MAbs	5989-0071EN	Application Note
Determination of the Glycosylation Status of Intact Recombinant Human Antibodies using Time of Flight Mass Spectrometry	Poroshell 300	MAbs	N/A	Technical Poster
High Speed and Ultra-High Speed Peptide Mapping of Human Monoclonal IgG on Poroshell 300SB-C18, C8, and C3	Poroshell 300	MAbs	5989-0590EN	Application Note
Rapid HPLC Analysis of Monoclonal Antibody IgG1 Heavy Chains using ZORBAX Poroshell 300SB-C8	Poroshell 300	MAbs	5989-0070EN	Application Note
Comparison of ZORBAX StableBond 300Å LC Columns to Optimize Selectivity for Antibody Separations Using HPLC and LC/MS	ZORBAX 300SB	MAbs	5989-6840EN	Application Note
Ultra High Speed and High Resolution Separations of Reduced and Intact Monoclonal Antibodies with Agilent ZORBAX RRHD Sub-2 µm 300 Diphenyl UHPLC Column	ZORBAX RRHD 300-Diphenyl	MAbs	5990-9668EN	Application Note
Reversed-Phase Optimization for Ultra Fast Profiling of Intact and Reduced Monoclonal Antibodies using Agilent ZORBAX Rapid Resolution High Definition 300SB-C3 Column	ZORBAX RRHD 300SB-C3	MAbs	5990-9667EN	Application Note

(Continued)

BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Reversed-Phase Separation of Intact Monoclonal Antibodies (MAb) using Agilent ZORBAX RRHD 300SB-C8	ZORBAX RRHD 300SB-C8	MAbs	5990-9016EN	Application Note
Rapid UHPLC Analysis of Reduced Monoclonal Antibodies using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 Column	ZORBAX RRHD 300SB-C8	MAbs	5990-9631EN	Application Note
Increased UV-Sensitivity in Combination with Novel WCX Column Separation for Better Detectability of Charge State Variants of Biotherapeutic Proteins	Bio MAb	MAbs and other proteins	N/A	Technical Poster
Agilent HPLC Column Selection Guide	HPLC columns	Many	5990-4435EN	Selection Guide
The LC Handbook: Guide to LC Columns and Method Development	LC columns	Method development	5990-7595EN	Primer
Agilent PLRP-S 100Å HPLC Columns and Media	PLRP-S	Oligonucleotides	5990-8187EN	Flyer
HPLC Purification of 26-bp Serial Analysis of Gene Expression Ditags	PLRP-S	Oligonucleotides	5990-7739EN	Application Note
Improved Column Lifetime with Thermally Stable Polymer Columns for Oligonucleotide Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7764EN	Application Note
Ion-Pair Reversed-Phase Purification of De-Protected Oligonucleotides – Choice of Pore Size	PLRP-S	Oligonucleotides	5990-7763EN	Application Note
Use Temperature to Enhance Oligonucleotide Mass Transfer and Improve Resolution in Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7765EN	Application Note
High Resolution Separations of Oligonucleotides using PL-SAX Strong Anion-Exchange HPLC Columns	PL-SAX	Oligonucleotides	5990-8297EN	Application Note
Fast Impurity Profiling of Synthetic Oligonucleotides with the Agilent 1290 Infinity LC System and Agilent 6530 Accurate-Mass QTOF LC/MS	ZORBAX Eclipse Plus C18 RRHD	Oligonucleotides	5990-5825EN	Application Note
Agilent PLRP-S Media and Load & Lock Columns – The Future of Prep/Process Chromatography	Prep/Process	Oligonucleotides, Peptides, Proteins	5990-8201EN	Flyer
Agilent PLRP-S 50 µm HPLC Media	PLRP-S	Oligonucleotides, Peptides, Small proteins	5990-8188EN	Flyer

(Continued)

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Analysis of Peptides on a PLRP-S 100Å 10 µm with ELS Detection and Acetonitrile-Free Eluents	PLRP-S	Peptides	5990-7760EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides	PLRP-S	Peptides	5990-7740EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a SepTech ST150 10-C18	SepTech	Peptides	5990-7951EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a VariTide RPC	VariTide RPC	Peptides	5990-8145EN	Application Note
Fast Monitoring of Bacteriophage Production During Fermentation Using the Agilent Bio-Monolith HPLC Column	Bio-Monolith	Phage production, process monitoring	5990-3247EN	Application Note
Physicochemical Characterization of a Therapeutic Protein by Peptide Mapping, SEC and IEX using the Agilent 1260 Infinity Bio-inert Quaternary LC System	Bio MAb, Bio SEC, ZORBAX Eclipse Plus, Poroshell 120	Protein analysis	5990-6192EN	Application Note
Optimization of the Agilent 1100 HPLC System for Superior Results with ZORBAX Poroshell Columns	Poroshell 300	Protein analysis	5988-9998EN	Application Note
Using Poroshell 300SB-C18 for High-Sensitivity, High-Throughput Protein Analysis on the Agilent LC/MSD	Poroshell 300-C18	Protein analysis	5988-7031EN	Application Note
Analysis of Albumin Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7852EN	Application Note
Analysis of Complex Bacterial Cell Division Proteins by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8143EN	Application Note
Analysis of Globulins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7851EN	Application Note
Analysis of Hsp47, a Collagen Chaperone, by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8142EN	Application Note
Analysis of Various Globular Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7850EN	Application Note
Effect of pH on Protein Size Exclusion Chromatography	ProSEC 300S	Protein analysis	5990-8138EN	Application Note
Globular Proteins and the Calibration of ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7767EN	Application Note

(Continued)

BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Reduce Tubing Volume to Optimize Column Performance	Small diameter columns	Optimizing instrument performance	5990-4964EN	Application Note
Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS	ZORBAX 300 Extend-C18	Optimizing instrument performance	5989-0683EN	Application Note
Increase Sensitivity with Microbore Polymeric HPLC Columns from Agilent	PLRP-S (Microbore)	Peptide hormone, small proteins, small molecules	5990-8666EN	Technical Overview
Decreasing Analysis Time Using Poroshell 300SB-C18 in Analysis of a Protein Digest	Poroshell 300	Peptide mapping	5988-6081EN	Application Note
Rapid Peptide Mapping Method with High Resolution using a sub 2-µm Column	ZORBAX 300SB-C18	Peptide mapping	5990-4712EN	Application Note
Increased Peak Capacity for Peptide Analysis with the Agilent 1290 Infinity LC System	ZORBAX Eclipse Plus	Peptide mapping	5990-6313EN	Application Note
Trypsin-Digested Monoclonal Antibody and BSA using Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Peptide mapping	5990-8244EN	Application Note
Preparative Scale Purification of Bradykinin by Concentration Overload	PLRP-S	Peptide purification	5990-7736EN	Application Note
Preparative Scale Purification of Bradykinin by Volume Overload	PLRP-S	Peptide purification	5990-7741EN	Application Note
Preparative Scale Purification of Depherelin by Concentration Overload	PLRP-S	Peptide purification	5990-7742EN	Application Note
Preparative Scale Purification of Leuprolide by Concentration Overload	PLRP-S	Peptide purification	5990-7735EN	Application Note
Superior Resolution of Peptides on SepTech ST150 10-C18 using Acetonitrile-Free Gradient Elution	SepTech	Peptide purification	5990-7761EN	Application Note
Agilent PLRP-S Media for HPLC Analysis of Peptides	PLRP-S	Peptides	5990-8667EN	Technical Overview

(Continued)

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Light Scattering Analysis of BSA with ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7766EN	Application Note
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7939EN	Application Note
LC Handbook and Compliance Guide to Recombinant Protein Characterization	N/A	Protein analysis	5990-8561EN	Primer
Agilent ZORBAX 300SB-C18 1.8µm Rapid Resolution High Definition Columns for Proteins	ZORBAX 300SB-C18	Protein analysis	5990-7989EN	Technical Overview
Analysis of Oxidized Insulin Chains using Reversed-Phase Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Protein analysis	5990-7988EN	Application Note
Fast Separation of Recombinant Human Erythropoietin using Reversed-Phase Agilent ZORBAX RRHD 300SB-C18, 1.8 µm	ZORBAX RRHD 300SB-C18	Protein analysis	5990-9248EN	Application Note
ACN-free HPLC Analysis and Prep Purification of ACP Fragment	PLRP-S	Protein purification	5990-7762EN	Application Note
Isocratic Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Protein purification	5990-7737EN	Application Note
Agilent PL-SAX Anion-Exchange Media for Amyloglucosidase Purification and Analysis	PL-SAX	Protein purification	5990-8664EN	Technical Overview
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Protein purification	5990-8141EN	Application Note
Optimizing Protein Separations with Agilent Weak Cation-Exchange Columns	Bio IEX	Protein separation	5990-9628EN	Application Note
Faster Separations Using Agilent Weak Cation-Exchange Columns	Bio IEX	Protein separation	5990-9931EN	Application Note
Optimum Pore Size for Characterizing Biomolecules with Agilent Bio SEC Columns	Bio SEC	Protein separation	5990-9894EN	Application Note
Separation of High MW Fibrous Proteins	PLRP-S	Protein separation	5990-8137EN	Application Note

(Continued)

BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Fast Protein Separations Using Agilent Poroshell 300	Poroshell 300	Protein separation	5989-9899EN	Application Note
Fast Separation of Large and Heterogeneous Proteins using ZORBAX Poroshell C18, C8, and C3 Phases	Poroshell 300	Protein separation	5989-0015EN	Application Note
Protein Identification and Impurity Profiling using Wide-Pore Reversed-Phase HPLC/UHPLC	Poroshell 300	Protein separation	5991-0625EN	Brochure
Use of Temperature to Increase Resolution in the Ultrafast HPLC Separation of Proteins with ZORBAX Poroshell 300SB-C8 HPLC Columns	Poroshell 300-C8	Protein separation	5989-0589EN	Application Note
The Effect of NaCl Concentration on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8139EN	Application Note
The Effect of Temperature on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8140EN	Application Note
Infinitely Better for Bio-Molecule Analysis	Agilent 1260 Infinity Bio-inert Quaternary LC System	Proteins	5990-6220EN	Brochure
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8832EN	Technical Poster
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8895EN	Application Note
Compliance for Biopharmaceutical Laboratories	Many	Proteins	5990-7001EN	Primer
Gradient Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Proteins	5990-7738EN	Application Note
Fast Agilent HPLC for Large Biomolecules	PLRP-S, PL-SAX, PL-SCX	Proteins	5990-8663EN	Technical Overview
Agilent Anion-Exchange Media for Proteins – Loading vs Resolution – Effect of Flow Rate and Example Protein Separations	PL-SAX	Proteins	5990-8777EN	Technical Overview
Purity Assessment Following Affinity Separation	PL-SAX	Proteins	5990-8436EN	Technical Overview
Agilent PL-SCX Cation-Exchange Media for Large Biomolecules	PL-SCX	Proteins	5990-8665EN	Technical Overview

(Continued)

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Poroshell 300SB-C18 for Fast, High Protein Separation	Poroshell 300	Proteins	5988-2100ENUS	Brochure
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Proteins	5990-8141EN	Application Note
ProSEC 300S Columns Protein Characterization Columns	ProSEC 300S	Proteins	5990-7468EN	Flyer
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Proteins	5990-7939EN	Application Note
Confidently Separate and Characterize Biomolecules with Agilent BioHPLC Columns	Bio SEC, Bio IEX, Bio MAb	Proteins	5990-5195EN	Brochure
Increase your Productivity with Agilent ZORBAX RRHD 300Å 1.8 µm Columns	ZORBAX RRHD 300SB-C18, C8	Proteins, Peptides	5990-8124EN	Flyer
High Purity, High Recovery, High Throughput – Agilent Technologies Offers Two New Lines of Preparative HPLC Columns	Agilent Prep HT	Purification/Prep	5989-2350EN	Brochure
Biomolecule Purification – Purification Columns and Media for Peptides, Oligonucleotides, and Proteins	PLRP-S, PL-SAX, PL-SCX	Purification/Prep	5990-8335EN	Brochure
The Influence of Silica Pore Size on Efficiency, Resolution and Loading in Reversed-Phase HPLC	SepTech	Purification/Prep	5990-8298EN	Application Note
Analysis of Protein Primary Structure when using Wide-Pore sub-2-µm Particles and UHPLC	ZORBAX RRHD 300SB-C18	Purification/Prep	5990-8830EN	Technical Poster
Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns	ProSEC 300S	SEC	5990-8147EN	Application Note

TIPS & TOOLS

For the latest application notes and new product information, go to **www.agilent.com/chem/library**

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GPC/SEC Columns and Standards

- A full portfolio of products for analysis of synthetic and natural polymers
- A wide selection of polymer standards to cover the range of applications in organic and water based solvents
- PL aquagel-OH-series, for aqueous SEC separations, and PLgel, for organic polymer applications, are available in mixed and individual pore sizes across a range of particle sizes, to cover the full spectrum of molecular weights (MW)
- Prep scale columns are available, along with narrow bore columns and columns designed for specific applications

Gel permeation chromatography (GPC) and size exclusion chromatography (SEC) are names applied to the most popular technique for measuring the molecular weight distribution (MWD) of natural and synthetic polymers, a property that affects many of the physical parameters of materials such as strength, toughness and chemical resistance. GPC and SEC are liquid chromatographic techniques that separate individual polymer chains on the basis of their size in solution and not on their chemistry. Gel permeation chromatography (GPC) is the name used to describe the analysis of polymers in organic solvents, such as tetrahydrofuran. Size exclusion chromatography (SEC) is the name used to describe the analysis of polymers in water and water-based solvents, such as buffer solutions. GPC/SEC is the only established method for obtaining a comprehensive understanding of a polymer's molecular weight distribution.

TIPS & TOOLS

For information on SEC columns for proteins, turn to pages 416-417.

Table of Contents

LC and LC/MS Columns for GPC/SEC

GPC/SEC Columns

The key to successful GPC/SEC separations is the correct choice of columns. The comprehensive range of Agilent products for GPC/SEC has been designed to cover virtually all polymer analysis application areas, and to make selection for the correct column, solvent, and calibration standard fast and reliable.

Agilent's PLgel GPC series of columns are for polymer applications using organic solvents. PLgel is a highly cross-linked, porous polystyrene/divinylbenzene matrix, which is recognized as a market leader in GPC column technology. PLgel materials have high pore volume and high-efficiency to maximize resolution. Their unequalled solvent compatability makes for easy transfer between polar and non-polar eluents, and outstanding physical rigidity provides extended lifetimes that maximize downtime. For more information and full ordering details, see pages 496-497.

Agilent's PL aquagel-OH series of columns provide a chemically and physically stable matrix for reliable aqueous SEC separations. The columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality. The "neutral" surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of compounds with neutral, ionic, and hydrophobic moieties, alone or in combination. PL aquagel-OH is available for analytical and preparative applications. For more information and full ordering details, see page 523.

Polymer standards for GPC/SEC

Agilent manufactures the highest quality polymer standards with extremely narrow polydispersity and the widest molecular weight range commercially available. These quality polymer standards are supplied with extensive characterization data utilizing a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign the peak molecular weight (Mp).

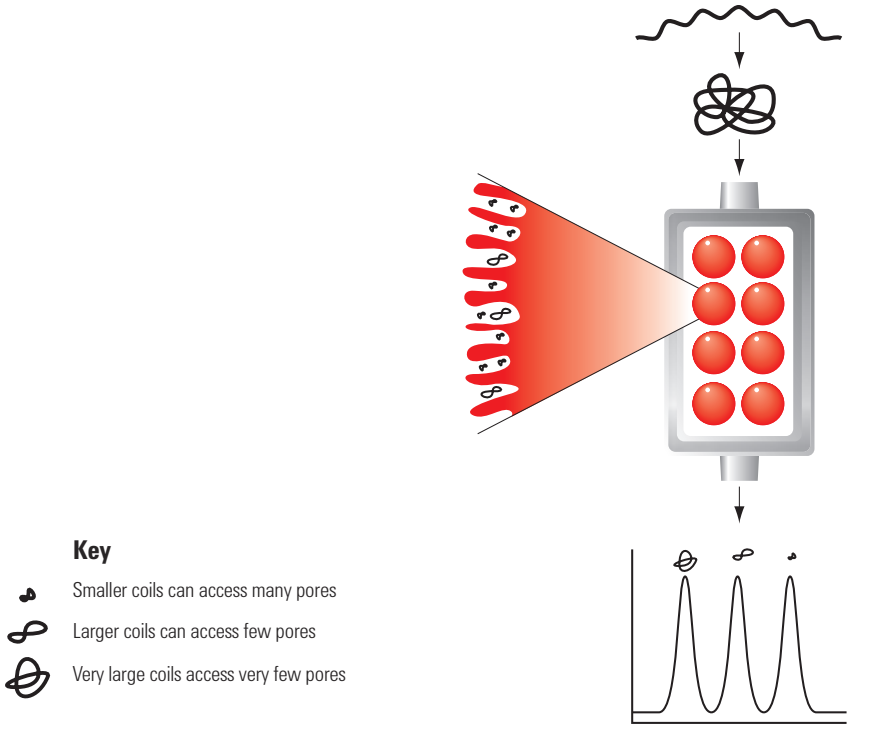
EasiVial – for organic and aqueous calibration. EasiVial is the fastest and most convenient method to deliver an accurate 12-point column calibration. EasiVial eliminates tedious weight procedures for improved calibration accuracy and reduces solvent dispensing to limit risks associated with handling solvents.

EasiCal – for organic solvents. EasiCal packs are pre-prepared for a no-fuss process. Two different combs, each with ten detachable spatulas, support a mixture of five polymer standards. The cost-effective format is designed to save money.

Individual standards and kits – an extensive range of polymer standard kits of different chemistries designed to match specific column sets are available, as well as individual standards in various pack sizes. For more details about Agilent's calibration standards for GPC/SEC, see page 530.

How GPC/SEC works:

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- The polymer coils are introduced to eluent flowing through the column
- Columns are packed with insoluble porous beads with well-defined pore structure
- The size of pores is similar to that of the polymer coils
- The polymer coils diffuse in and out of the pores
- Result is elution based on size – large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards



Mechanisms of GPC and SEC

Recommendations for setting up a GPC/SEC system

The following questions will help you find the recommended columns and standards for any given application, as well as system parameters such as injection volumes.

Choosing an eluent for GPC/SEC

Question	Answer	Recommendation	Comments
1. What is the sample soluble in?	Water or water buffer with up to 50% methanol	Agilent PL aquagel-OH	Best choice for water-based applications but cannot accommodate organics apart from methanol up to 50%
Many polymers are only soluble in a small number of solvents. This is the key question when developing methods for analyzing polymers. The solvents mentioned here are all common eluents employed in GPC/SEC.	Typical organic solvent such as THF, chloroform, toluene	Agilent PLgel or Agilent PlusPore	PLgel are the workhorse columns, PlusPore columns are an alternative
	Organic/water mixtures or polar organics such as, DMF, NMP	Agilent PolarGel	PolarGel is a smaller column range than PLgel or PL aquagel-OH columns but is suited to mixtures of organics and water

TIPS & TOOLS

More information on GPC/SEC instrumentation and systems is a click away. We have a variety of application notes, data sheets and brochures available from Agilent for free.

To learn more, visit
www.agilent.com/chem/gpc

Choosing a column for GPC/SEC

Columns shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
2. What is the expected molecular weight? *It may seem strange to ask this question, but in GPC/SEC the resolution of a column is related to the resolving range. Knowing something of the expected molecular weight of a sample helps to choose the best column that will give optimum results.*	High (up to several millions)	Aqueous solvents **PL aquagel-OH MIXED-H 8 µm** or combination of PL aquagel-OH 40 and 60 15 µm	The 15 µm column combination is best only where sample viscosity is very high, otherwise 8 µm columns give greater resolution
		Organic solvents **PLgel 10 µm MIXED-B** or PLgel 20 µm MIXED-A	The PLgel MIXED-A column resolves higher than the PLgel MIXED-B but at lower efficiency due to larger particle size
		Mixed solvents **PolarGel**	No PolarGel column available for this molecular weight range. Contact your local GPC/SEC expert for advice
	Intermediate (up to hundreds of thousands)	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	A wide-ranging column that covers most water-soluble polymers
		Organic solvents **PLgel 5 µm MIXED-C** or PLgel 5 µm MIXED-D, PolyPore or ResiPore	The PLgel columns are the most widely applicable for the majority of applications; PolyPore and ResiPore columns are alternatives
		Mixed solvents **PolarGel-M**	Covers most applications
	Low (up to tens of thousands)	Aqueous solvents **Combination of PL aquagel-OH 40 and PL aquagel-OH 30 8 µm**	These two columns in a combined set cover the low end of the molecular weight range
		Organic solvents **PLgel 3 µm MIXED-E** or MesoPore	The PLgel column provides high resolution and is designed for low molecular weight applications; the MesoPore column is an alternative
		Mixed solvents **PolarGel-L**	For low molecular weight applications
	Very low (a few thousand)	Aqueous solvents **PL aquagel-OH 20 5 µm**	This high-performance column gives high resolution at low molecular weight
		Organic solvents **OligoPore** or PLgel 3 µm 100Å	The OligoPore column is less prone to dispersion than the PLgel column, but both work well
		Mixed solvents **PLgel**	No PolarGel column covers this range so use PLgel columns as alternatives
	Unknown	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	Covers the molecular weight ranges of most polymer samples
		Organic solvents **PLgel 5 µm MIXED-C** or PolyPore	This PLgel column is the most widely applicable for the majority of applications
		Mixed solvents **PolarGel-M**	Covers the majority of applications

Setting up the GPC/SEC system

Question	Answer	Recommendation	Comments
3. How many columns to use? *The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution and the more columns are required to maintain the quality of the results. For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples during analysis.*	Depends on the particle size of the columns	Particle size 20 µm use 4 columns Particle size 13 µm use 3 columns Particle size 10 µm use 3 columns Particle size 8 µm use 2 columns Particle size 5 µm use 2 columns Particle size 3 µm use 2 columns	Increased number of columns required for large particle sizes to make up for low efficiencies

Question	Answer	Recommendation	Comments
4. What size injection volume? *The injection volume required is dependent on the particle size of the column – smaller particles need lower injection volumes to minimize dead volume. Larger injection volumes allow the introduction of high molecular weight samples at lower concentrations, reducing viscosity and ensuring a quality chromatogram is obtained.*	Depends on the particle size of the columns	Particle size 20 µm use 200 µL injection Particle size 13 µm use 200 µL injection Particle size 10 µm use 200 µL injection Particle size 5 µm use 100 to 200 µL injection Particle size 3 µm use 20 µL injection	Smaller particle sizes require smaller loops to minimize band broadening

What standards should I use?

Standards shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
5. What is the eluent? *Standards are polymers, so the choice of standard mainly reflects solubility in the chosen eluents.*	Water or water buffer with up to 50% methanol	**Polyethylene glycol (PEG)/oxide (PEO)** or polysaccharides (SAC)	These standards perform in all water-based systems, PEG/PEO in convenient Agilent EasiVial format
	Typical organic solvent such as THF, chloroform, toluene	**Polystyrene (PS)** or polymethylmethacrylate (PMMA)	Polystyrene is the most commonly used standard in convenient EasiVial format
	Organic/water mixtures or polar organics such as DMF, NMP	**Polyethylene glycol/oxide** or polymethylmethacrylate	Polar standards perform well

(Continued)

What standards should I use?

Question	Answer	Recommendation	Comments
6. What format of standards are recommended?	For the quickest and simplest approach where accurate concentrations are not required	Easiest option – **EasiVial** or EasiCal	Simple to use, EasiVial preferred before EasiCal because of the wider choice of polymer types
Different formats of standards are available depending on customer preference.	If accurate concentrations are required	Accurate concentrations required – **EasiVial** or individual standards	Both formats allow accurate sample concentrations, EasiVials are simpler to use

Typical polymer molecular weights

If you are unsure of the molecular weight of your sample, the table below shows some approximate molecular weight ranges for common polymers, which will help you select the right column for your application.

Polymer Type	Typical molecular weight of polymer	Typical polydispersity[1] of polymer
Polymers from free radical synthesis	High (up to several million) Intermediate (up to hundreds of thousands)	~ 2
Polymers from ionic synthesis	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 1.01
Polymers from addition synthesis	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 2
Polymers from controlled radical polymerization	Low (up to tens of thousands) Very low (a few thousand)	~ 1.1 to 1.5
Polyolefins	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 200
Acrylates	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2
Small molecule additives	Very low (a few thousand)	1
Pre-polymers	Low (up to tens of thousands) Very low (a few thousand)	~ 2 to 10
Resins	Low (up to tens of thousands) Very low (a few thousand)	~ 2 to 10
Natural biopolymers such as polysaccharides	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 10
Rubbers	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 10
Biodegradable polymers	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 1.1 to 2

[1] Polydispersity is a measure of the distribution of molecular mass of a polymer. Polydispersity index (PDI) = M_w/M_n.

Organic GPC

PLgel GPC Columns

- Robust performance under the most exacting conditions
- Temperature stability up to 220 °C
- Solvent compatibility allows easy and rapid transfer between solvents of varying polarity

PLgel materials have high pore volume and high efficiency to maximize resolution. Their unequalled solvent compatibility makes for easy transfer between polar and non-polar eluents, and outstanding physical rigidity provides extended lifetimes that minimize downtime.

The key to successful GPC separations is the correct choice of columns. The comprehensive range of PLgel products has been designed to cover virtually all organic solvent-based polymer analysis application areas, and to make selection of the correct column, solvent, and calibration standard fast and reliable.

PLgel is a highly cross-linked, porous polystyrene/divinylbenzene matrix, which is recognized as a market leader in GPC column technology. PLgel is manufactured to ISO 9001:2000 and benefits from comprehensive QC/QA for total reproducibility, batch-to-batch and column-to-column.

Solvent Compatibility

PLgel columns are routinely supplied in ethyl benzene* but you can easily and rapidly transfer between solvents of varying polarity. In organic GPC, sample to column interaction may occur occasionally and eluent modification can be used to eliminate these effects. PLgel columns are the ideal choice for such analyses, as they easily tolerate eluents in the pH range 1-14, as well as up to 10% water in a miscible organic solvent.

PLgel is compatible with all of these solvents

Solvent Polarity	Solvent
6.0	Perfluoroalkane
7.3	Hexane
8.2	Cyclohexane
8.9	Toluene
9.1	Ethyl acetate
9.1	Tetrahydrofuran (THF)
9.3	Chloroform
9.3	Methyl ethyl ketone (MEK)
9.7	Dichloromethane
9.8	Dichloroethene
9.9	Acetone
10.0	o-Dichlorobenzene (o-DCB)
10.0	Trichlorobenzene (TCB)
10.2	m-Cresol
10.2	o-Chlorophenol (o-CP)
10.7	Pyridine
10.8	Dimethyl acetamide (DMAc)
11.3	n-Methyl pyrolidone (NMP)
12.0	Dimethyl sulfoxide (DMSO)
12.1	Dimethyl formamide (DMF)

*We also provide a custom packing service in which columns can be shipped in specific solvents to provide extra convenience to our customers.

PLgel Frit Porosity

Media Type	Porosity (µm)
PLgel 3 µm	2
PLgel 5 µm	2
PLgel 10 µm	5
PLgel 20 µm	10

For PLgel column accessories ordering information please see page 529

PLgel MIXED Columns

The PLgel MIXED range greatly simplifies column selection for easy decision making. By using these mixed columns, you can eliminate mismatched column sets and spurious peaks for more reliable results. Every column contains a mixture of individual pore size materials, accurately blended to cover a specified broad range of molecular weight with a linear calibration to eliminate column mismatch. Simply add extra columns for even greater resolution.

Column Specifications

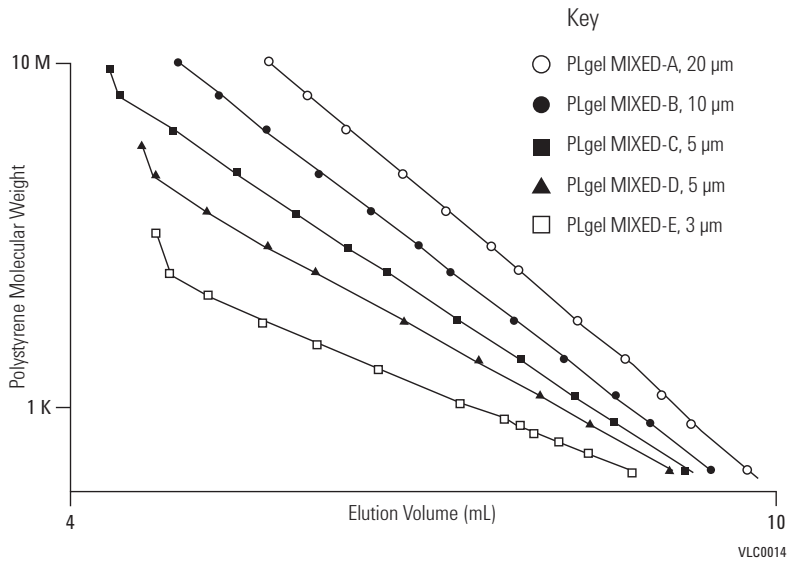
Column	Linear MW Operating Range (g/mol)	Guaranteed Column Efficiency	Typical Pressure	Maximum Flow Rate	Maximum Pressure	Maximum Temperature
PLgel MIXED-A	2,000-40,000,000	> 17,000 p/m	1 mL/min (7.5 mm id): ≈ 3 bar (44 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 2.4 bar (35 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	220 °C
PLgel MIXED-B	500-10,000,000	> 35,000 p/m	1 mL/min (7.5 mm id): ≈ 10 bar (145 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 8 bar (116 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	220 °C
PLgel MIXED-C	200-2,000,000	> 50,000 p/m	1 mL/min (7.5 mm id): ≈ 30 bar (435 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 24 bar (348 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	150 °C
PLgel MIXED-D	200-400,000	> 50,000 p/m	1 mL/min (7.5 mm id): ≈ 30 bar (435 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 24 bar (348 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	150 °C
PLgel MIXED-E	up to 30,000	7.5 x 300 mm: > 80,000 p/m 4.6 x 250 mm: > 70,000 p/m	1 mL/min (7.5 mm id): ≈ 50 bar (725 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 42 bar (609 psi) per 250 mm (THF @ 20 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	180 bar (2611 psi)	110 °C

PLgel MIXED Column Selection Guide

UHMW polymer distributions	PLgel MIXED-A, 20 µm
High MW polymers, demanding eluents	PLgel MIXED-B, 10 µm
Mid range MW polymers, high resolution	PLgel MIXED-C, 5 µm
Resins, condensation polymers	PLgel MIXED-D, 5 µm
Low MW resins, prepolymers	PLgel MIXED-E, 3 µm

PLgel MIXED Gel Calibration Curves

MIXED gel calibration curves are designed to be linear over a specified molecular weight range, ensuring that the same degree of resolution is achieved across the full operating range of the column. The particle size of the packing and porosity of a particular MIXED gel column are carefully matched to the MW range and application, thus optimizing performance and eliminating the effects of shear degradation. Resolution in GPC is controlled by the slope of the calibration curve and the particle size of the packing material. Agilent has scientifically determined the minimum number of MIXED gel columns required to perform accurate MWD determinations based on specific resolution (Rsp). Thus you can have complete confidence in the accuracy and precision of the calculated data.



Reference: Meehan, E. (1998) Size exclusion chromatography columns from Polymer Laboratories. In: Chi-San Wu (Ed.) Column Handbook for Size Exclusion Chromatography. Academic Press, New York, USA.

PLgel MIXED Columns

Description	Size (mm)	Part No.
PLgel 20 µm MIXED-A	7.5 x 300	PL1110-6200
PLgel 10 µm MIXED-B	7.5 x 300	PL1110-6100
PLgel 5 µm MIXED-C	7.5 x 300	PL1110-6500
PLgel 5 µm MIXED-D	7.5 x 300	PL1110-6504
PLgel 3 µm MIXED-E	7.5 x 300	PL1110-6300

PLgel MIXED Guards

Size (mm)	Particle Size (µm)	Part No.
7.5 x 50	20	PL1110-1220
7.5 x 50	10	PL1110-1120
7.5 x 50	5	PL1110-1520
7.5 x 50	3	PL1110-1320

Starches

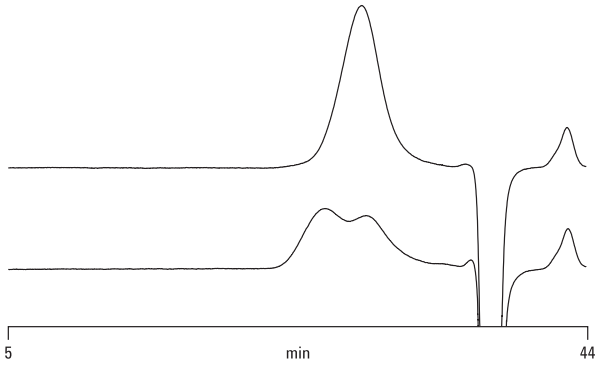
Column: **4 x PLgel 20 µm MIXED-A**
PL1110-6200
7.5 x 300 mm

Mobile Phase: DMSO + 5 mM $NaNO_3$

Flow Rate: 1.0 mL/min

Temperature: 80 °C

Detector: RI



Polyphenylene Sulfides

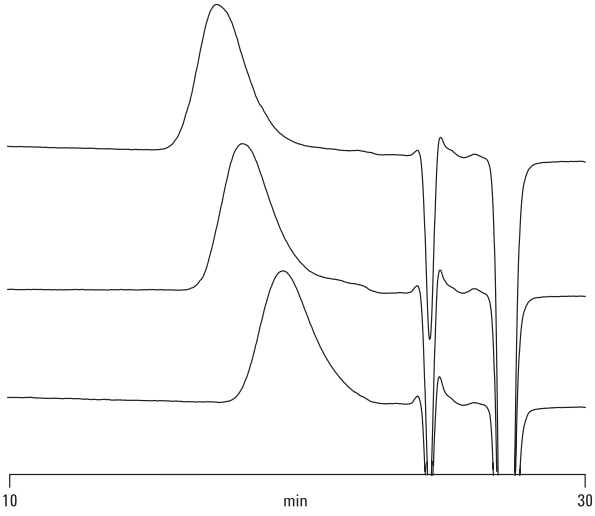
Column: **3 x PLgel 10 µm MIXED-B**
PL1110-6100
7.5 x 300 mm

Mobile Phase: o-Chloronaphthalene

Flow Rate: 1.0 mL/min

Temperature: 210 °C

Detector: RI



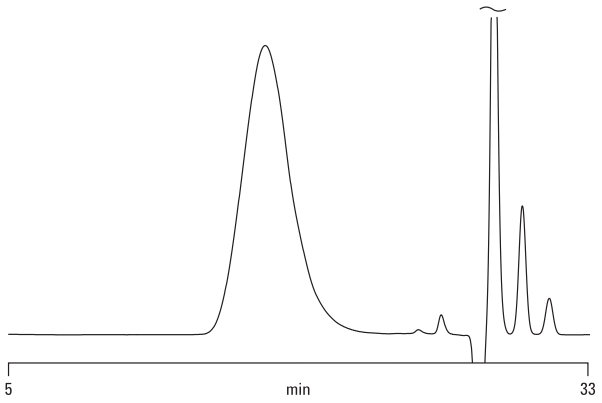
Plasticized PVC

Column: **3 x PLgel 5 µm MIXED-C**
PL1110-6500
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: RI



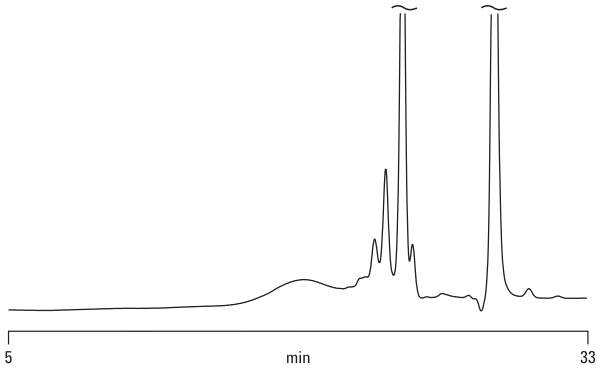
Epoxy Resin

Column: **3 x PLgel 5 µm MIXED-D**
PL1110-6504
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: RI



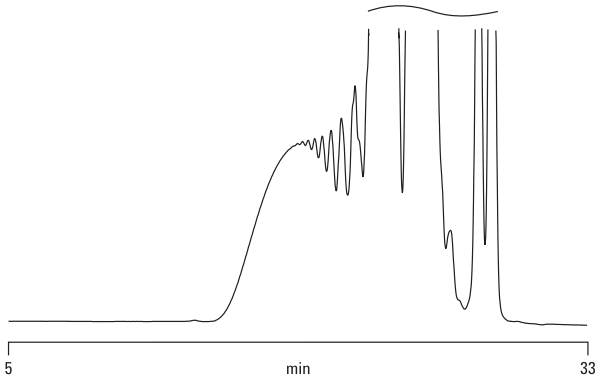
Polyol

Column: **PLgel 3 µm MIXED-E**
PL1110-6300
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm



PLgel MIXED-LS Columns

- Obtain an instant improvement in data quality
- No need for conditioning, saving time and solvent costs
- Maximize the potential of light scattering detectors

The PLgel MIXED-LS series is a PS/DVB packing using an innovative proprietary suspension polymerization technique to virtually eliminate nano-particle leakage. A startling improvement is achieved immediately in the quality of light scattering data obtained with PLgel MIXED-LS columns in place of conventional GPC columns. The light scattering chromatograms shown here were obtained after flushing the columns for one hour in THF at 1 mL/min. A polystyrene standard (Mp 210,000) was injected at 1 mg/mL in order to illustrate the dramatic improvement in signal-to-noise with the PLgel MIXED-LS column.

The performance of PLgel MIXED-LS columns has been matched to PLgel 20 µm MIXED-A and PLgel 10 µm MIXED-B columns in terms of calibration, column efficiency, wide solvent compatibility, and operating temperature. MIXED-LS are also ideal for online viscosity detection, minimizing the risk of capillary blockage, and can be used with regular PLgel guard columns that are packed with rigid low pore size gels with no particle bleed.

PLgel MIXED-LS Columns

Description	Size (mm)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
PLgel 10 µm MIXED-B LS	7.5 x 300	500-10,000,000	>35,000	PL1110-6100LS
PLgel 10 µm guard	7.5 x 50			PL1110-1120
PLgel 20 µm MIXED-A LS	7.5 x 300	2,000-40,000,000	>17,000	PL1110-6200LS
PLgel 20 µm guard	7.5 x 50			PL1110-1220

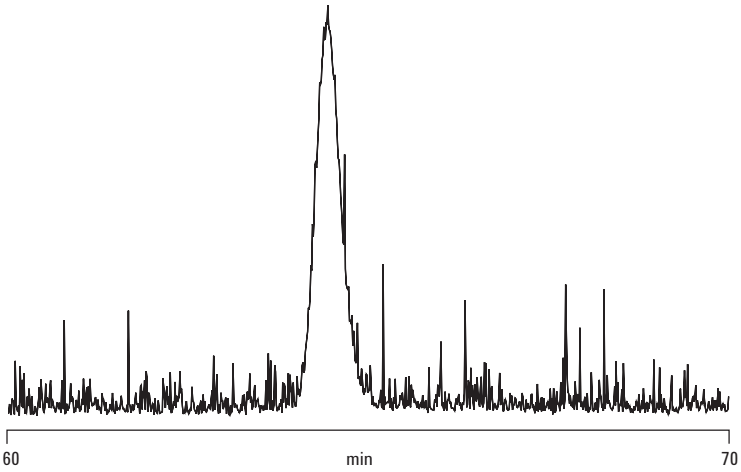
Conventional GPC column

Column: **Conventional GPC column**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: LS



Light scattering detection with a conventional GPC column – noise due to particulate bleed.

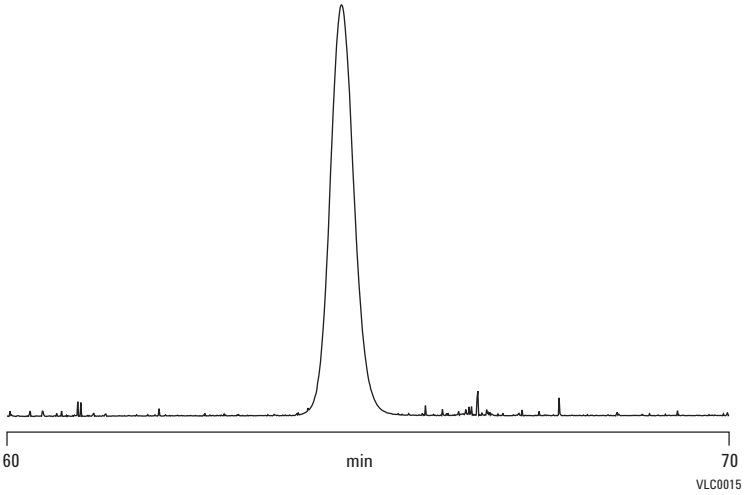
PLgel LS column

Column: **PLgel 10 µm MIXED-B LS**
PL1110-6100LS
7.5 x 300 mm, 10 µm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: LS



Light scattering detection with a PLgel LS column – minimal particulate bleed gives greatly improved baseline.

PLgel MiniMIX Columns

- Use about 70% less solvent and save money
- Store less solvent and increase operator safety
- High performance comparable to Agilent's conventional id columns

For reduced solvent cost and consumption, use industry standard PLgel MiniMIX mixed gel columns in 250 x 4.6 mm narrow bore dimensions. These narrow bore columns offer high performance, excellent solvent compatibility and mechanical stability. PLgel MiniMIX columns can be used with conventional GPC equipment.

To maintain the same linear velocity through the column, the volumetric flow rate must be reduced to 0.3 mL/min in line with the column cross sectional area, resulting in significantly lower solvent consumption. Sample loading should also be scaled down in line with reduced column volume, and system dead volume should be minimized to avoid excessive band broadening.

PLgel MiniMIX Columns

Description	Size (mm)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
PLgel 20 µm MiniMIX-A	4.6 x 250	2,000-40,000,000	> 17,000	PL1510-5200
PLgel 20 µm MiniMIX-A guard	4.6 x 50			PL1510-1200
PLgel 10 µm MiniMIX-B	4.6 x 250	500-10,000,000	> 35,000	PL1510-5100
Plgel 10 µm MiniMIX-B guard	4.6 x 50			PL1510-1100
PLgel 5 µm MiniMIX-C	4.6 x 250	200-2,000,000	> 50,000	PL1510-5500
PLgel 5 µm MiniMIX-C guard	4.6 x 50			PL1510-1500
PLgel 5 µm MiniMIX-D	4.6 x 250	200-400,000	> 50,000	PL1510-5504
PLgel 5 µm MiniMIX-D guard	4.6 x 50			PL1510-1504
PLgel 3 µm MiniMIX-E	4.6 x 250	up to 30,000	> 70,000	PL1510-5300
PLgel 3 µm MiniMIX-E guard	4.6 x 50			PL1510-1300

PLgel Individual Pore Size Columns

- Very high efficiency improves productivity
- Choose the optimum column for a perfect match of performance and application
- Fast analysis with fewer columns saves time and money

Individual pore size GPC columns offer high resolution over a specific molecular weight range. The linear portion of the calibration curve, where the slope is at its shallowest, defines the MW region over which optimum resolution will be achieved.

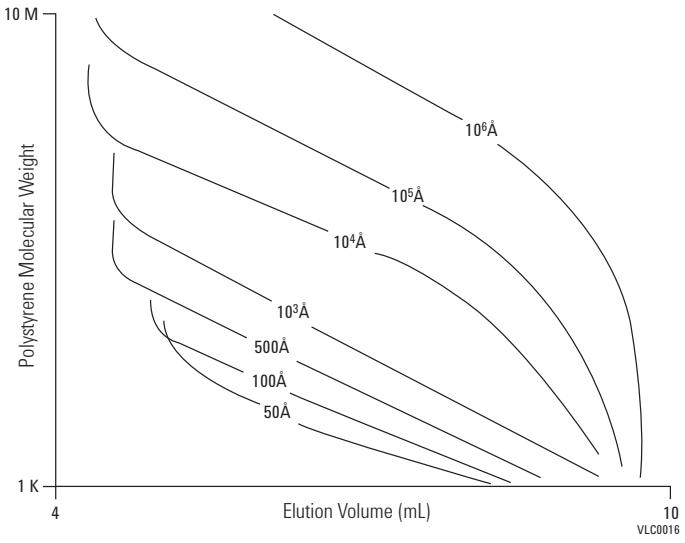
PLgel Individual Pore Size Columns

Size (mm)	Particle Size (µm)	Pore Size (Å)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
7.5 x 300	3	100	up to 4,000	> 100,000	PL1110-6320
7.5 x 300	5	50	up to 2,000	> 60,000	PL1110-6515
7.5 x 300	5	100	up to 4,000	> 60,000	PL1110-6520
7.5 x 300	5	500	500-30,000	> 60,000	PL1110-6525
7.5 x 300	5	10^3	500-60,000	> 50,000	PL1110-6530
7.5 x 300	5	10^4	10,000-600,000	> 50,000	PL1110-6540
7.5 x 300	5	10^5	60,000-2,000,000	> 50,000	PL1110-6550
7.5 x 300	10	50	up to 2,000	> 35,000	PL1110-6115
7.5 x 300	10	100	up to 4,000	> 35,000	PL1110-6120
7.5 x 300	10	500	500-30,000	> 35,000	PL1110-6125
7.5 x 300	10	10^3	500-60,000	> 35,000	PL1110-6130
7.5 x 300	10	10^4	10,000-600,000	> 35,000	PL1110-6140
7.5 x 300	10	10^5	60,000-2,000,000	> 35,000	PL1110-6150
7.5 x 300	10	10^6	600,000-10,000,000	> 35,000	PL1110-6160

PLgel Guard Column information can be found on page 500

Calibration curves

Calibrant: Polystyrene
Mobile Phase: THF
Flow Rate: 1.0 mL/min



PLgel Preparative Columns

- Excellent column efficiency provides optimum resolution
- High loading can isolate mg amounts for further study
- Over 10 times scale up permits efficient quantification

Preparative GPC is generally employed to fractionate polymers, isolate components in a polymer formulation or simplify mixtures of relatively small molecules in complex matrices. Mixtures of materials are easily separated on the basis of size, preferably in a low boiling organic solvent. They are then collected as a series of discrete fractions and isolated by simple evaporation of the solvent.

PLgel preparative columns are packed with the same rigid, high performance media as the analytical columns. The 10 µm particle provides high column efficiency (> 25,000 p/m) for optimum resolution and loading characteristics. PLgel 25 mm id preparative columns offer over 10 times scale-up compared to the 7.5 mm analytical columns. The increased id and column volume permit even higher loading. With low molecular weight materials, sample concentration can also be significantly increased, enabling production of milligram quantities of very pure material. The actual loading is ultimately controlled by the sample and its molecular weight.

PLgel Preparative Columns

Size (mm)	Particle Size (µm)	Pore Size (Å)	Linear MW Operating Range (g/mol) (PS)	Part No.
25 x 300	10	50	up to 2,000	PL1210-6115
25 x 300	10	100	up to 4,000	PL1210-6120
25 x 300	10	500	500-30,000	PL1210-6125
25 x 300	10	10^3	500-60,000	PL1210-6130
25 x 300	10	10^4	10,000-600,000	PL1210-6140
25 x 300	10	10^5	60,000-2,000,000	PL1210-6150
25 x 300	10	10^6	600,000-10,000,000	PL1210-6160
MIXED-B 25 x 300	10		500-10,000,000	PL1210-6100
MIXED-D 25 x 300	10		200-400,000	PL1210-6104
Prep guard 25 x 25				PL1210-1120

Columns for Special GPC/SEC Applications

EnviroPrep

- High sample loading ensures effective trace analysis
- Simple clean-up procedure saves sample preparation costs
- Optimized particle size distribution provides high resolution

EnviroPrep columns permit a simple, one stage clean-up as part of a methodology to determine pesticides in many organic matrices. The higher molecular weight fractions such as lipids, polymers, natural resins and dispersed high molecular weight components are easily eliminated in the GPC analysis.

Preparative GPC for soil extract clean-up is described in EPA Method 3640A using 300 x 25 mm and 150 x 25 mm columns to give higher sample loading and fraction yields, which is particularly useful for low levels of pollutants. Low pore size EnviroPrep columns are ideal for this method. The columns have 10 µm particles with 100Å pore sizes for high resolution, with an exclusion limit of 4000 g/mol. The preparative columns offer good resolution and high loading through optimization of the particle size distribution.

EnviroPrep

Size (mm)	Part No.
21.2 x 150	PL1E10-3120EPA
25 x 150	PL1210-3120EPA
21.2 x 300	PL1E10-6120EPA
25 x 300	PL1210-6120EPA

Columns for sample clean-up

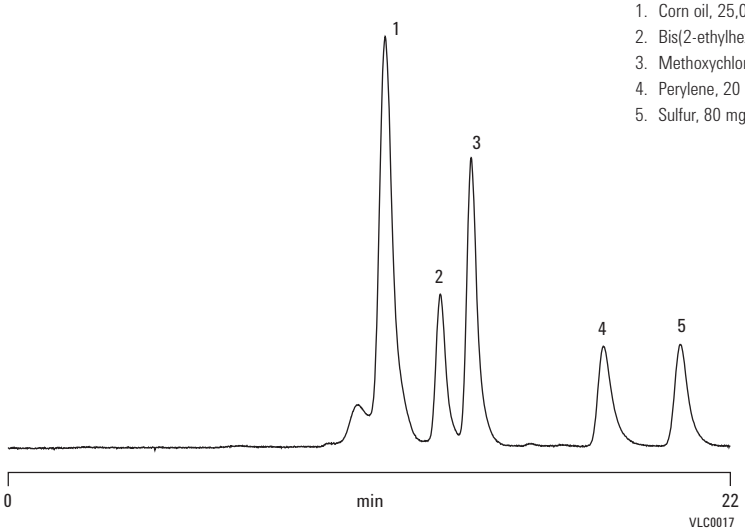
Column: **EnviroPrep PL1210-6120EPA 25 x 300 mm**

Column: **EnviroPrep PL1210-3120EPA 25 x 150 mm**

Mobile Phase: DCM

Flow Rate: 10 mL/min

Detector: UV, 254 nm



1. Corn oil, 25,000 mg/L
2. Bis(2-ethylhexyl) phthalate, 1,000 mg/L
3. Methoxychlor, 200 mg/L
4. Perylene, 20 mg/L
5. Sulfur, 80 mg/L

PLgel Olexis

- Optimized design for polyolefin analysis
- High temperature capability
- High resolution with no damage from sample shear provides clean separations

PLgel Olexis is designed for the analysis of very high molecular weight polymers, specifically polyolefins. The column resolves up to 100,000,000 g/mol (polystyrene in THF), and is packed with 13 µm particles to optimize efficiency and resolution without the risk of sample shear degradation during analysis. The packing of PLgel Olexis has the mechanical stability and robustness expected from a PLgel column, and so it is able to operate up to 220 °C for the analysis of highly crystalline materials.

PLgel Olexis

Description	Size (mm)	Part No.
PLgel Olexis	7.5 x 300	PL1110-6400
PLgel Olexis guard	7.5 x 50	PL1110-1400

PLgel Olexis reveals true modalities across the range of polyolefins

Column: **3 x PLgel Olexis PL1110-6400 7.5 x 300 mm**

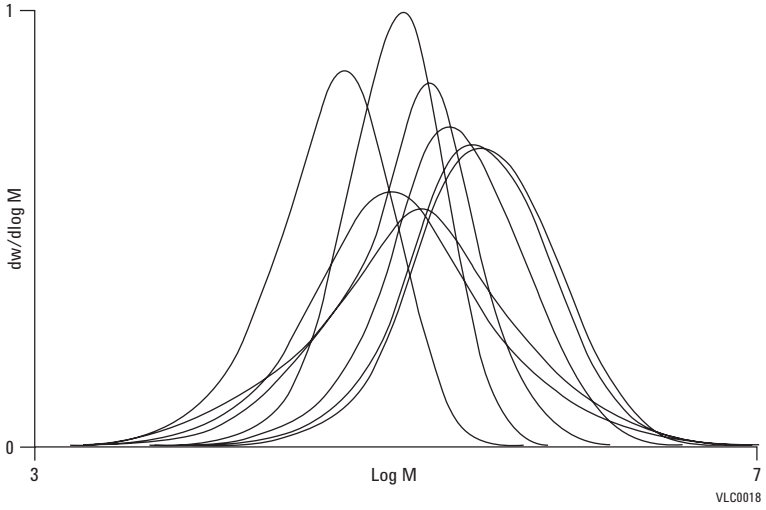
Mobile Phase: Trichlorobenzene + 0.0125% BHT

Flow Rate: 1.0 mL/min

Injection Volume: 200 µL

Temperature: 160 °C

Detector: PL-GPC 220 (RI)



PL HFIPgel

- Optimized separation range delivers high performance with no artifacts
- Highly durable packing prolongs column lifetime
- Low operating pressure reduces system wear and unnecessary downtimes

Hexafluoroisopropanol (HFIP) is used as a solvent in GPC for the analysis of important industrial polymers such as polyesters, polyamides and polylactide/glycolide copolymers. For greatly improved performance in extremely polar solvents such as HFIP and trifluoroethanol, we have developed novel "multipore" technology to produce PL HFIPgel, a PS/DVB packing featuring a monodisperse particle size, high pore volume, and high resolution.

Using PL HFIPgel avoids issues associated with conventional packings and HFIP, such as excessive curvature of calibration curves, dislocations/shoulders on peaks for polydisperse samples, and poor resolution in the low MW region.

Column efficiency is guaranteed > 30,000 p/m and the columns are very durable, with a maximum operating pressure of 145 bar (2030 psi). They are packed and tested in methanol but shipped ready-to-use in HFIP.

PL HFIPgel columns with 7.5 mm id normally operate at 1 mL/min. However, the 4.6 mm id columns run at 0.3 mL/min, providing a 70% reduction in solvent consumption with consequent savings in the cost of buying and disposing of solvents.

MW range for PL HFIPgel columns is 2,000,000 g/mol (PMMA in THF).

PL HFIPgel

Description	Size (mm)	Part No.
PL HFIPgel	4.6 x 250	PL1514-5900HFIP
PL HFIPgel	7.5 x 300	PL1114-6900HFIP
PL HFIPgel guard	7.5 x 50	PL1114-1900HFIP
PL HFIPgel guard	4.6 x 50	PL1514-1900HFIP

Polyamides

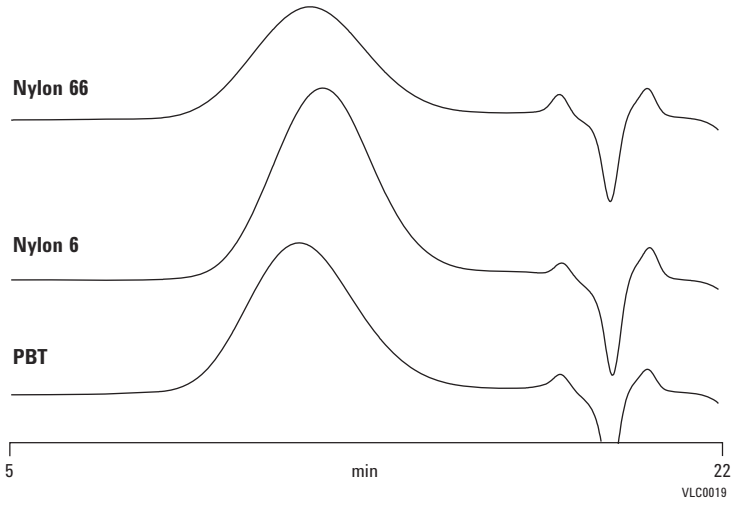
Column: **2 x PL HFIPgel PL1114-6900HFIP 7.5 x 300 mm**

Mobile Phase: HFIP + 20mM NaTFAc

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: PL-GPC 50 (RI)



PL Rapide

- Analysis in less than ten minutes saves time
- Significantly increased sample throughput improves efficiency
- Reduced solvent consumption and disposal costs save money
- Available in L, M, and H versions for low, medium, and high molecular weights; available in F version for flow injection analysis

Rapid GPC is an excellent tool for screening polymer MWD for trend analysis. Short PL Rapide columns reduce analysis times while maintaining the excellent solvent compatibility and mechanical stability of all GPC columns from Agilent.

PL Rapide columns are ideal for high speed applications such as high throughput screening, process monitoring, or tracking changes in MW distributions, where time is the most critical factor in the analysis. Packed with high quality gels, these columns cover the complete spectrum of molecular weights and are available for the analysis of both organic and water soluble polymers. Key features include high pore volume, high resolution packing materials, no special system requirements, choice of molecular weight resolving range, wide solvent compatibility, and excellent mechanical stability.

PL Rapide

Description	Size (mm)	MW Range (g/mol)	Guaranteed Efficiency (p/m)	Part No.
PL Rapide H	7.5 x 150	500-10,000,000	> 35,000	PL1113-3100
	10 x 100			PL1013-2100
PL Rapide M	7.5 x 150	200-2,000,000	> 60,000	PL1113-3500
	10 x 100			PL1013-2500
PL Rapide L	7.5 x 150	200-400,000	> 80,000	PL1113-3300
	10 x 100			PL1013-2300
PL Rapide F	7.5 x 150	up to 4,500	> 55,000	PL1113-3120
	10 x 100	up to 4,500	> 40,000	PL1013-2120
PL Rapide Aqua H	7.5 x 150	100-10,000,000	> 35,000	PL1149-3800
	10 x 100			PL1049-2800
PL Rapide Aqua L	7.5 x 150	100-30,000	> 35,000	PL1120-3830
	10 x 100			PL1020-2830

Resin analysis by rapid GPC

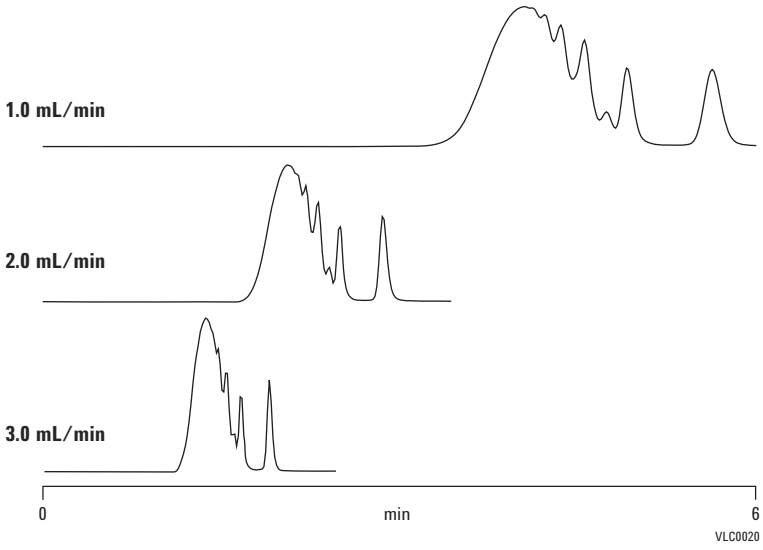
Column: **PL Rapide L**
PL1013-2300
10 x 100 mm

Sample: Epoxy resin

Mobile Phase: THF

Flow Rate: 1.0 , 2.0 and 3.0 mL/min

Detector: UV, 254 nm



PolarGel

- Medium polarity surface and high mechanical stability
- Operate in a wide range of solvents and solvent combinations
- Available in two resolving ranges, PolarGel-L and PolarGel-M

The PolarGel range is ideal for use with polar solvents, such as dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO), and for solvent combinations such as tetrahydrofuran with water. These eluents are very useful in GPC/SEC to separate polar materials, such as polar resins, modified polysaccharides or complex polar polymers that are difficult to analyze in traditional SEC solvents, such as tetrahydrofuran alone. PolarGel-L is used for low molecular weight polar polymers and PolarGel-M for high MW polar polymers.

With polar polymers, highly polar groups can lead to non-specific interactions and secondary separation mechanisms when using polar solvents and traditional non-polar styrene/divinylbenzene columns. Additives and/or column conditioning are normally required to reduce these interactions. PolarGel has no need for these interventions, and also avoids the interactions and secondary effects that produce chromatogram distortions.

These PolarGel "mixed bed" columns have a medium polarity surface and high mechanical stability. They are capable of operating in a wide range of solvents and solvent combinations, greatly enhancing your ability to analyze polar polymers that are not necessarily water soluble. PolarGel is available in two resolving ranges to meet your precise requirements.

PolarGel

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Part No.
PolarGel-L	7.5 x 300	Up to 30,000	PL1117-6830
PolarGel-L guard	7.5 x 50		PL1117-1830
PolarGel-L repair gel			PL1417-0830
PolarGel-M	7.5 x 300	Up to 2,000,000	PL1117-6800
PolarGel-M guard	7.5 x 50		PL1117-1800
PolarGel-M repair gel			PL1417-0800

Two samples of melamine resin analyzed by PolarGel-L

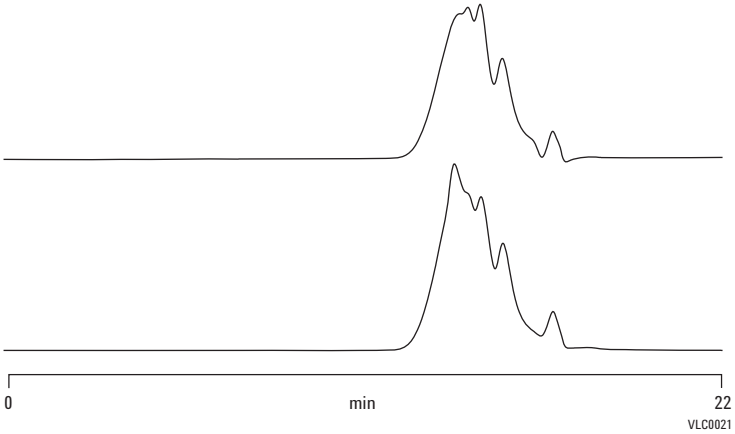
Column: **2 x PolarGel-L, 300 x 7.5 mm PL1117-6830**

Mobile Phase: Dimethyl acetamide + 0.1% LiBr

Flow Rate: 1.0 mL/min

Injection Volume: 100 µL

Detector: Agilent PL-GPC 220 (RI)



Excellent separation of two phenol formaldehyde resins with PolarGel-M

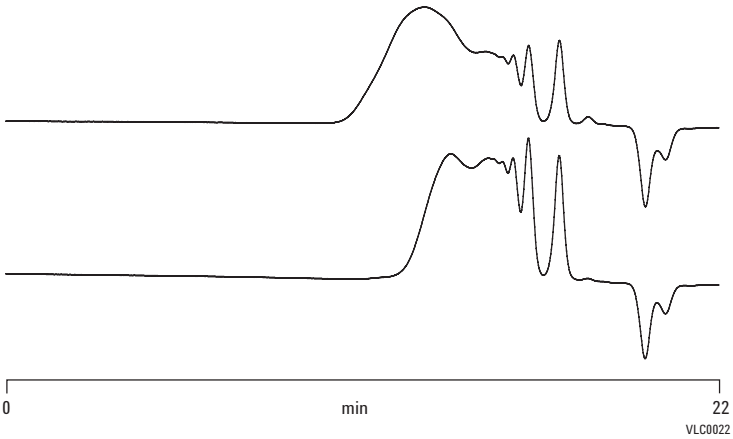
Column: **2 x PolarGel-M, 300 x 7.5 mm PL1117-6800**

Mobile Phase: 0.2% (w/v) DMF & 0.1% LiBr to reduce sample aggregation

Flow Rate: 1.0 mL/min

Injection Volume: 100 µL

Detector: Agilent PL-GPC 50 (RI)



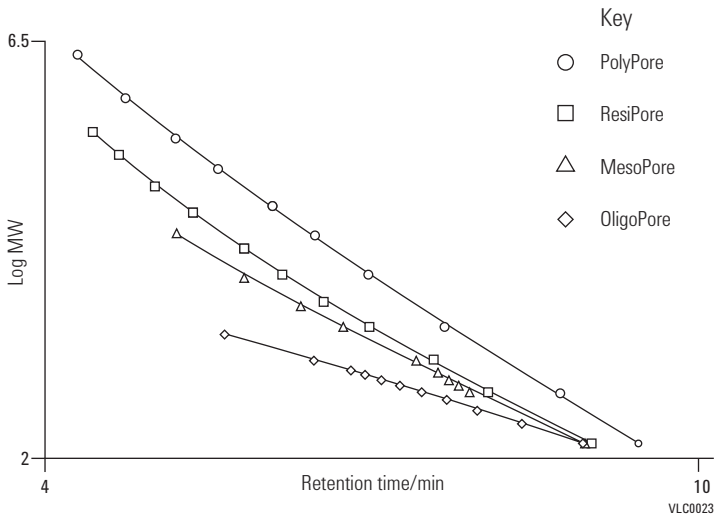
PlusPore

The PlusPore range has an increased pore volume that provides high resolution for specific applications. The high stability media permits the use of a wide range of organic solvents with accuracy and precision so that there is no distortion of the MW distribution shape.

The PlusPore series of columns has been specifically designed for high resolution GPC, and represents the very latest in GPC column technology. These novel packing materials are based on the industry standard, highly cross-linked polystyrene/divinylbenzene (PS/DVB), for the widest applicability and solvent compatibility. Each is made using a novel polymerization process to produce particles that exhibit a specific, controlled pore structure for optimum GPC performance. Typical applications include resins, condensation polymers, prepolymers, and oligomers.

For high resolution polymer analysis, the PolyPore, ResiPore, MesoPore, and OligoPore columns of the PlusPore product series exhibit a wide pore size distribution with near linear calibration curves covering an extended molecular weight range. These so-called "multipore" structures have increased pore volume compared to regular PS/DVB packing materials. This results in very high resolution GPC columns designed for specific application areas. The highly cross-linked porous particles provide excellent chemical and physical stability and permit easy transfer across the full range of organic solvents with little change in the shape of the calibration curve or the efficiency of the columns. As this multipore column technology does not require the combination of individual pore size packing materials, the result is high accuracy and precision without any artifacts in the shape of the molecular weight distribution.

PlusPore calibration curves



PlusPore Selection Guide

Column	MW Range (g/mol) (PS)	Nominal Particle Size (µm)	Typical Efficiency (p/m)	Recommended Calibrants	Frit Porosity (µm)
PolyPore	200-2,000,000	5	> 60,000	EasiCal PS-1or EasiVial PS-H	2
ResiPore	200-400,000	3	> 80,000	EasiCal PS-2 or EasiVial PS-M	2
MesoPore	up to 25,000	3	> 80,000	Polystyrene S-L-10 Kit	2
OligoPore	up to 4,500	6	> 55,000	Polystyrene S-L2-10 Kit	2

PolyPore

- Routine polymer analysis with very high resolution
- Wide operating range simplifies column choice
- Low particle size extracts maximum information from the analyte

PolyPore columns have been specifically developed to give unrivaled resolution for the analysis of polymers with broad molecular weight distributions. With a wide operating range covering many decades of molecular weight, PolyPore columns combine a 5 µm particle size with extremely high pore volume to give the highest possible resolution, ensuring the most detailed information possible from your analysis.

PolyPore

Description	Size (mm)	Part No.
PolyPore	7.5 x 300	PL1113-6500
PolyPore guard	7.5 x 50	PL1113-1500

Comparison of PolyPore with conventional individual pore size GPC columns

Column A: **2 x PolyPore PL1113-6500 7.5 x 300 mm**

Column B: **PLgel 10^3Å 7.5 x 300 mm, 5 µm**

Column C: **PLgel 10^5Å 7.5 x 300 mm, 5 µm**

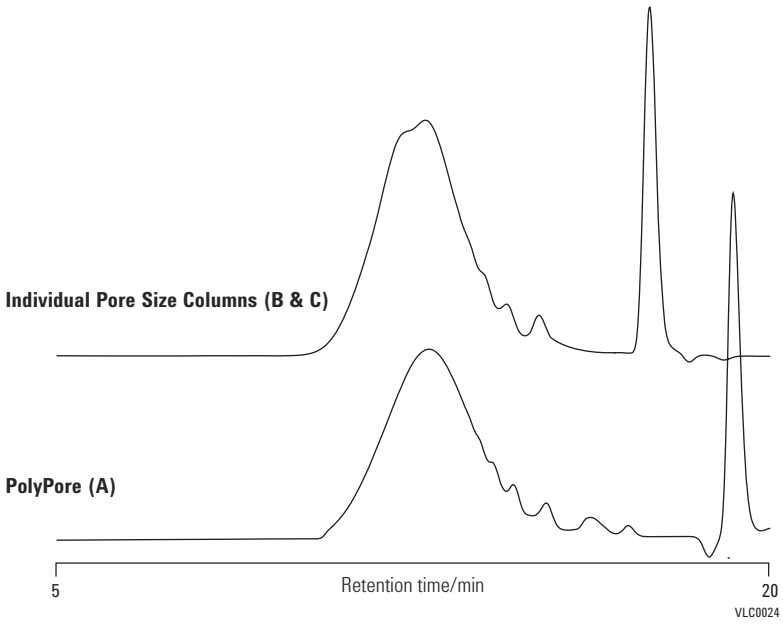
Sample: High MW Resin

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume 100 µL

Detector: Agilent PL-GPC 50 (RI)



Polymethylmethacrylate in DMF

Column: **2 x PolyPore PL1113-6500 7.5 x 300 mm**

Sample: Commercial PMMA

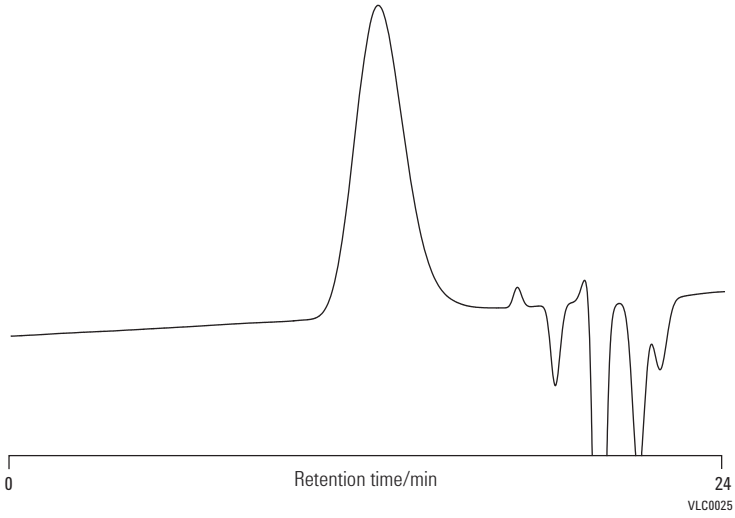
Mobile Phase: DMF + 0.1% LiBr

Flow Rate: 1.0 mL/min

Temperature: 80 °C

Injection Volume: 100 µL

Detector: Agilent PL-GPC 50 (RI)



ResiPore

- Efficient separation of complex molecular weight distributions
- Reveals oligomer content to provide a true representation of the sample
- High pore volume extracts maximum information from the analyte

ResiPore columns are the ideal choice for the analysis of resins and condensation polymers with complex molecular weight distributions that include oligomer content. By combining a 3 µm particle size and high pore volume, high efficiency ResiPore columns offer maximum resolution of these intermediate molecular weight polymers.

ResiPore

Description	Size (mm)	Part No.
ResiPore	7.5 x 300	PL1113-6300
ResiPore guard	7.5 x 50	PL1113-1300

Alkyd resin

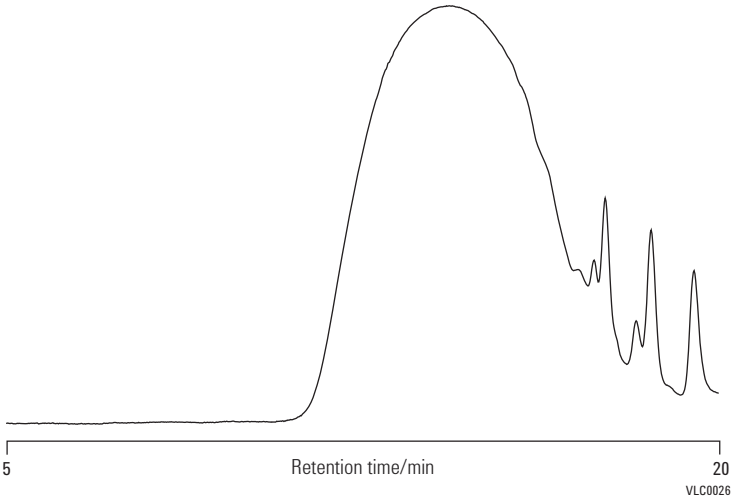
Column: **2 x ResiPore PL1113-6300 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: UV, 254 nm



Polyester

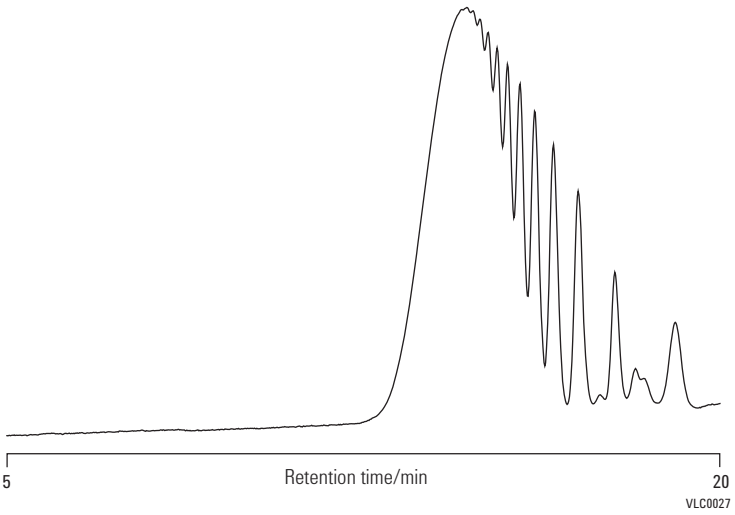
Column: **2 x ResiPore PL1113-6300 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: UV, 254 nm



MesoPore

- Full solvent compatibility with no detrimental effect on efficiency
- Low particle size extracts maximum information from the analyte
- No MWD dislocations so the distribution is an accurate representation of the sample

MesoPore columns have been specifically designed to provide optimum results in the analysis of prepolymers, i.e. polymeric materials with a large oligomeric component. By combining a 3 µm particle size with high pore volume, MesoPore columns give the highest resolution separations for the analysis of low molecular weight polymers, such as prepolymers, resins, polyols, and siloxanes.

MesoPore

Description	Size (mm)	Part No.
MesoPore	7.5 x 300	PL1113-6325
MesoPore guard	7.5 x 50	PL1113-1325

Polyurethanes

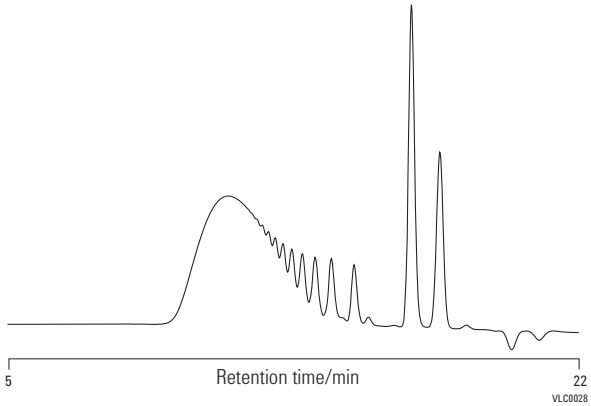
Column: **2 x MesoPore PL1113-6325 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: Agilent PL-GPC 50 (RI)



Polyesterimide

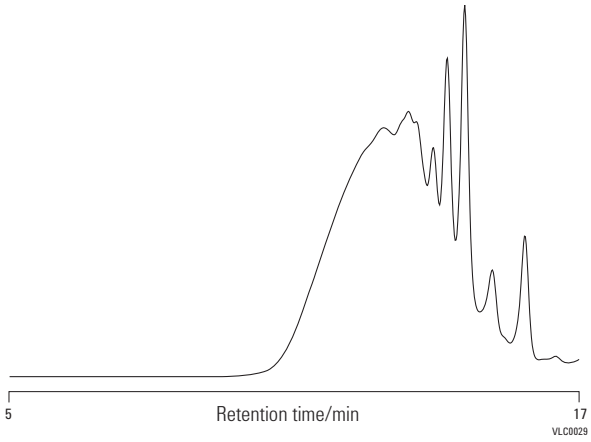
Column: **2 x MesoPore PL1113-6325 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: Agilent PL-GPC 50 (RI)



OligoPore

- Near linear calibration curve for best accuracy and precision
- Very stable media allows for a wide choice of solvents
- Isolation of individual fractions reveals more information from whole samples

OligoPore columns have been developed from an innovative new media that exhibits significantly increased pore volumes compared to conventional low pore size GPC columns. The outcome is higher resolution in the oligomeric region. The 300 x 25 mm preparative column offers high resolution at greatly increased loading for effective isolation of individual components. Oligomer fractions collected from the OligoPore preparative column can then be re-injected on analytical columns to check for the purity of the fractions and for comparison with the whole sample.

OligoPore

Description	Size (mm)	Part No.
OligoPore	25 x 300	PL1213-6520
OligoPore	7.5 x 300	PL1113-6520
OligoPore guard	7.5 x 50	PL1113-1320

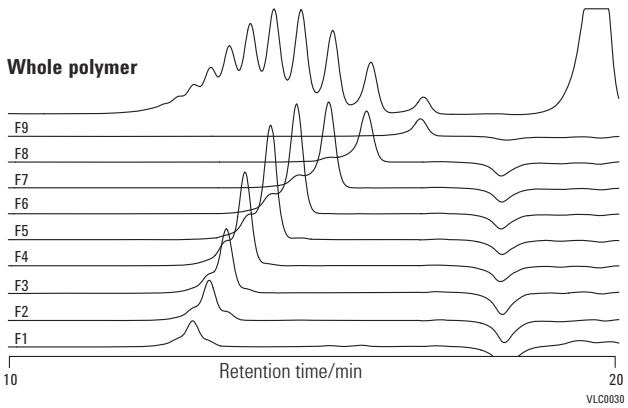
Analysis of low molecular weight polystyrene and oligomer fractions collected from OligoPore preparative columns

Column: **2 x OligoPore PL1113-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: UV



Analytical separation of low molecular weight polystyrene

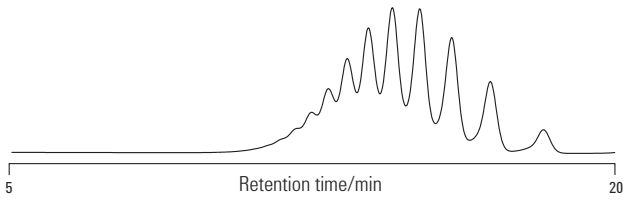
Column: **2 x OligoPore PL1213-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Loading: 0.2%, 100 mL

Detector: UV



Preparative separation of low molecular weight polystyrene

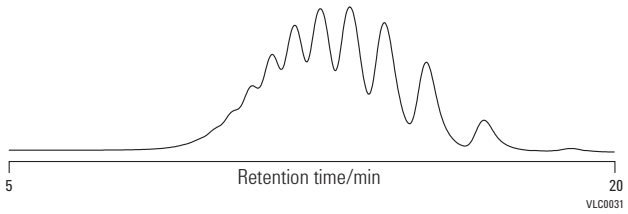
Column: **2 x OligoPore PL1213-6520 25 x 300 mm**

Mobile Phase: THF

Flow Rate: 10.0 mL/min

Loading: 2.0%, 2 mL

Detector: UV



Aqueous SEC of Polymers

PL aquagel-OH SEC

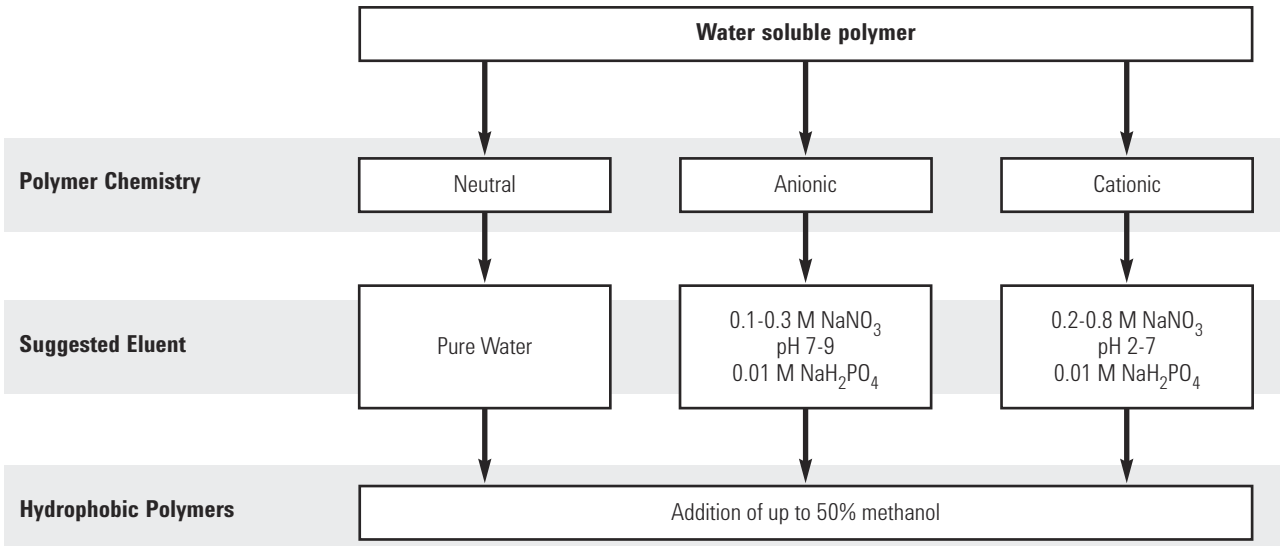
Aqueous size exclusion chromatography (SEC) is widely used for the determination of molecular weight distributions of a variety of synthetic and naturally occurring water-soluble polymers, and separations of oligomers and small molecules. The requirement to eliminate ionic and hydrophobic effects makes aqueous SEC very demanding.

The PL aquagel-OH series provides a chemically and physically stable matrix for reliable aqueous SEC separations. The columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality. The "neutral" surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of compounds with neutral, ionic, and hydrophobic moieties, alone or in combination. PL aquagel-OH is available for analytical and preparative applications.

Optimizing Conditions for Aqueous SEC with PL aquagel-OH Columns

Due to the complex nature of water-soluble polymers, it is often necessary to modify the eluent in order to avoid sample-to-sample and sample-to-column interactions which can result in poor aqueous SEC separations. The excellent stability of the PL aquagel-OH packing material allows the eluent to be tailored to suit the polymer, while retaining the high column efficiency. For ionic interactions, the eluent can be modified by the addition of salt and/or the adjustment of pH. For water soluble polymers with a hydrophobic character, only the addition of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions.

Guide to eluent selection for PL aquagel-OH applications



PL aquagel-OH Column Selection Guide

Sample Type	Typical Applications	Recommended Column Sets
Low MW polymers and oligomers	Surfactants, oligosaccharides, PEGs, lignosulfonates, polyacrylates	2 or 3, 30, 20 PL aquagel-OH 8 µm, or PL aquagel-OH 20 5 µm, or PL aquagel-OH MIXED-M 8 µm
Polydisperse synthetic or naturally occurring polymers	Polysaccharides, PVA, cellulose derivatives, PEO, polyacrylic acid	2 or 3 PL aquagel-OH MIXED-H 8 µm, or PL aquagel-OH 60/50/40 8 µm
Very high MW polymers	Polyacrylamides, hyaluronic acids, CMC, starches, gums	PL aquagel-OH 60/50/40 15 µm in series

PL aquagel-OH Analytical

- Highly stable matrix ensures reliable separations, even with modified eluents
- MIXED columns cover a wide range of molecular weights, simplifying column selection
- Highly versatile for neutral, polar, anionic and cationic samples

The PL aquagel-OH analytical series has a pH range of 2-10, compatibility with organic solvent (up to 50% methanol), mechanical stability up to 140 bar (2030 psi) and low column operating pressures.

PL aquagel-OH Analytical

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Guaranteed Efficiency (p/m)	Part No.
PL aquagel-OH 20 5 µm	7.5 x 300	100-20,000	> 5,000	PL1120-6520
PL aquagel-OH 20 8 µm	7.5 x 300	100-20,000	> 35,000	PL1149-6820
PL aquagel-OH 30 8 µm	7.5 x 300	100-30,000	> 35,000	PL1120-6830
PL aquagel-OH 40 8 µm	7.5 x 300	10,000-200,000	> 35,000	PL1149-6840
PL aquagel-OH 40 15 µm	7.5 x 300	10,000-200,000	> 15,000	PL1149-6240
PL aquagel-OH 50 8 µm	7.5 x 300	50,000-1,000,000	> 35,000	PL1149-6850
PL aquagel-OH 50 15 µm	7.5 x 300	50,000-1,000,000	> 15,000	PL1149-6250
PL aquagel-OH 60 8 µm	7.5 x 300	200,000-> 10,000,000	> 35,000	PL1149-6860
PL aquagel-OH 60 15 µm	7.5 x 300	200,000-> 10,000,000	> 15,000	PL1149-6260
PL aquagel-OH MIXED-H 8 µm	7.5 x 300	100-10,000,000	> 35,000	PL1149-6800
PL aquagel-OH MIXED-M 8 µm	7.5 x 300	Up to 600,000	> 35,000	PL1149-6801
PL aquagel-OH 10 µm guard	25 x 25			PL1249-1120
PL aquagel-OH 5 µm guard	7.5 x 50			PL1149-1530
PL aquagel-OH 8 µm guard	7.5 x 50			PL1149-1840

TIPS & TOOLS

Buffers in a stored column may crystallize and cause damage. Flush the column with water containing a small amount of sodium azide to prevent biological growth.

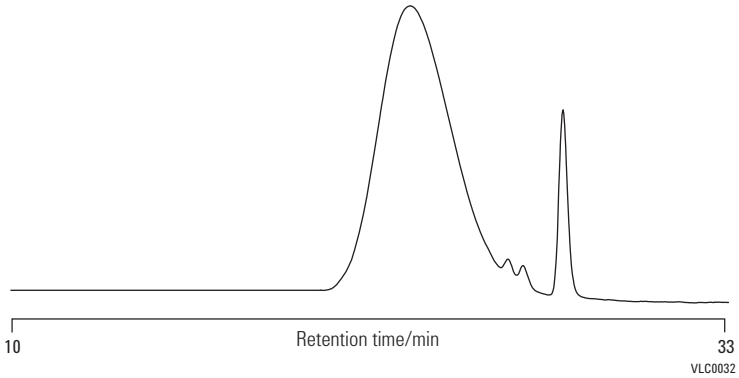
Polyvinyl alcohol

Column: **3 x PL aquagel-OH MIXED-H 8µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



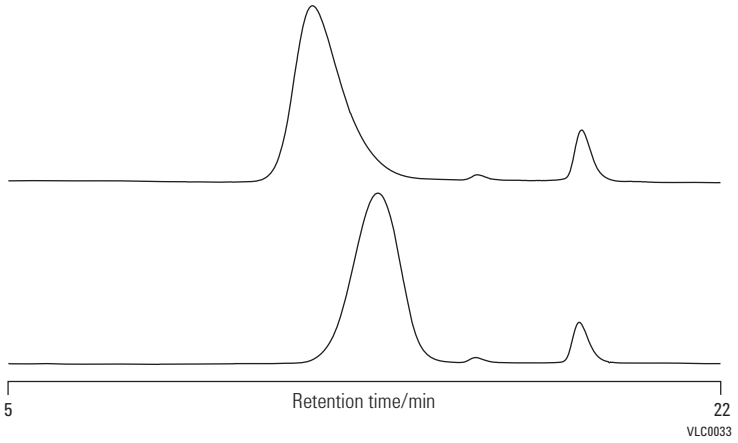
Heparin

Column: **2 x PL aquagel-OH 30 8 µm**
PL1120-6830
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Hyaluronic acid

Column: **PL aquagel-OH 60 15 µm**
PL1149-6260
7.5 x 300 mm

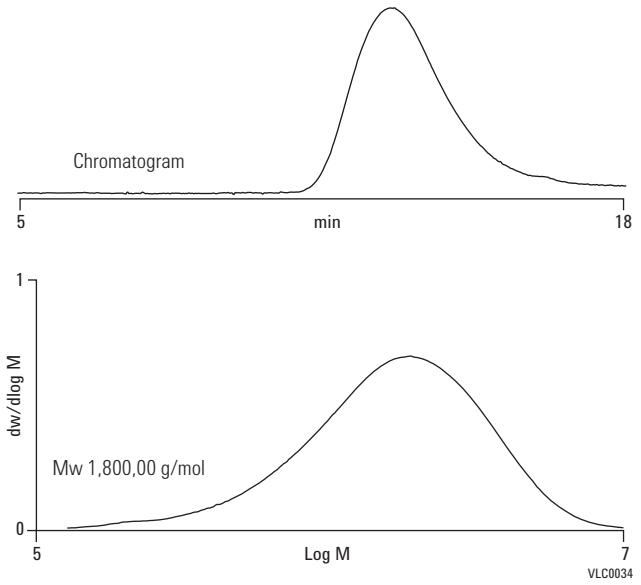
And

PL aquagel-OH 40 15 µm
PL1149-6240
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Differences in composition of two alkyl naphthalene sulfonates

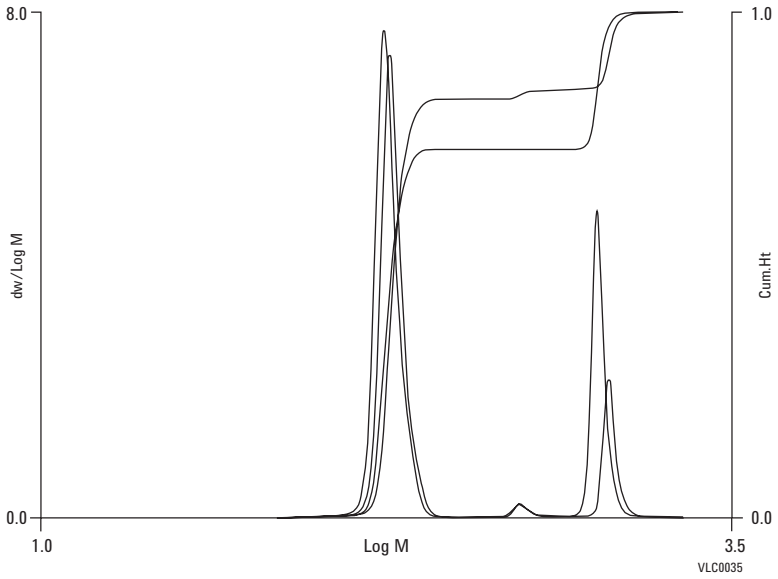
Column: **2 x PL aquagel-OH 20 5µm**
PL1120-6520
7.5 x 300 mm

Mobile Phase: 0.25 M ammonium formate in water

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: ELS (neb=30 °C, evap=30 °C, gas=1.4 SLM)



PL aquagel-OH Preparative

- Up to 10 times scale-up maximizes yield
- High loading maximizes sample throughput
- Carefully chosen particle size provides optimum resolution

Preparative SEC is used for the fractionation of a wide variety of water-soluble samples based on their size in solution. The technique is applied to the fractionation of disperse polymers or to isolate components in a polymer formulation.

Preparative PL aquagel-OH columns and associated guard columns enable rapid and convenient scale-up from analytical separations. The 25 mm id prep column offers at least a 10 times scale-up in loading from the 7.5 mm id analytical columns. Typically, a 10 mL/min flow rate results in a separation time of ten minutes with a 300 mm column. The columns are packed with the same robust macroporous particles as the analytical column range. The 8 µm particle size provides optimum resolution and loading characteristics with column efficiency > 20,000 plates/m.

PL aquagel-OH Preparative

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Part No.
PL aquagel-OH 30 8 µm	25 x 300	100-30,000	PL1220-6130
PL aquagel-OH 40 8 µm	25 x 300	10,000-200,000	PL1249-6140
PL aquagel-OH 50 8 µm	25 x 300	50,000-1,000,000	PL1249-6150
PL aquagel-OH MIXED 8 µm	25 x 300	100-10,000,000	PL1249-6100
PL aquagel-OH 10 µm guard	25 x 25		PL1249-1120

Polyvinyl alcohol

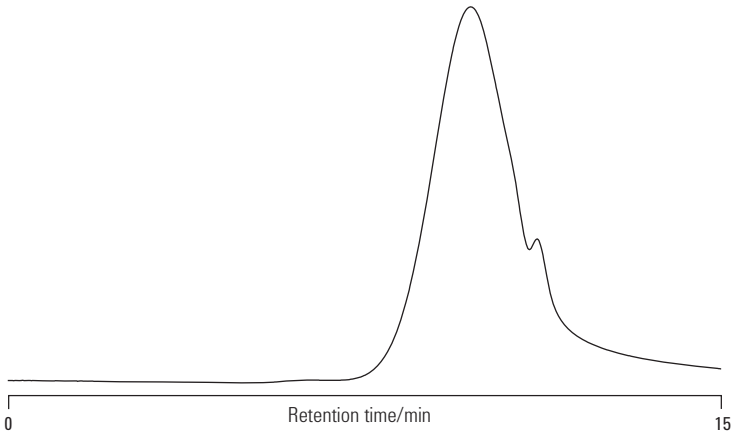
Column: **PL aquagel-OH 40 8 µm PL1249-6140 25 x 300 mm**

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 10.0 mL/min

Loading: 10 mg/mL, 2 mL

Detector: Agilent PL-GPC 50 (RI)



GPC Column Accessories

Description	Unit	Part No.
Frit removal tool for threaded columns only	1/pk	PL1310-0001
2 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0002
5 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0012
10 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0036
PLgel column repair gel, 10 µm	1/pk	PL1410-0101
PLgel column repair gel, 5 µm	1/pk	PL1410-0501
Column connecting nuts, 1/16 in tube	5/pk	PL1310-0007
Tubing ferrules, 1/16 in tube	5/pk	PL1310-0008
Connecting tubing, 10 cm length, 0.01 in id	10/pk	PL1310-0048
LDV intercolumn stainless steel connector	1/pk	PL1310-0005
PLgel column repair gel, 3 µm	1/pk	PL1410-0301
PLgel Olexis column repair gel	1/pk	PL1410-0200

Polymer Standards for GPC/SEC

Polymer standards from Agilent are the ideal reference materials for generating accurate, reliable GPC/SEC column calibrations, with the assurance of the ISO 9001:2000 quality standard. Additional applications for our highly characterized homopolymers exhibiting unique characteristics are used as model polymers for research and analytical method development.

Agilent manufactures the highest quality polymer standards with extremely narrow polydispersity and the widest molecular weight range commercially available. These quality polymer standards are supplied with extensive characterization data utilizing a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign that all important peak molecular weight (Mp).

Our comprehensive range of EasiVial, EasiCal, and traditional calibration kits has been specifically designed to cover all molecular weight ranges for organic and aqueous GPC/SEC applications. We provide you with the widest choice to maximize your specific characterization needs. In addition, we supply other polymers as individual molecular weights, and broad distribution polymers for system validation or broad standard calibration procedures.

Calibration Kits

Agilent offers a wide range of polymer standards kits for conventional GPC/SEC column calibration or for calibrating light scattering and viscometry detectors. The kits are in boxed sets of ten different polymer standards covering a particular molecular weight range, to be used with organic and aqueous, medium polarity, and polar solvents. Every individual polymer has its own Certificate of Analysis of the analytical conditions and values, such as Mp needed for constructing a calibration plot. The polymers are chosen to give equidistant calibration points on a logarithmic MW scale, providing a more uniform calibration curve.

Individual Polymer Molecular Weights

We design our individual standards to have the narrowest molecular weight distribution commercially available. Additionally, they cover the widest molecular weight range, from 162-15 million MW. The current polystyrene nominal molecular weight of 15 million MW has a polydispersity ≤1.10. These standards are generally available in 1, 5 and 10 g quantities, and each comes with its own Certificate of Analysis detailing analysis conditions and relevant data.

GPC/SEC Standards Selection Guide

Polymer Type	Individual MW	Calibration Kits	EasiCal	EasiVial	Type of GPC/SEC
Polystyrene	✓	✓	✓	✓	Organic
Polymethylmethacrylate	✓	✓		✓	Organic
Polyethylene glycol (PEG)	✓	✓		✓	Organic/Aqueous
Polyethylene oxide (PEO)	✓	✓		✓	Organic/Aqueous
Pullulan polysaccharide	✓	✓			Organic/Aqueous
Polyacrylic acid Na salt	✓	✓			Aqueous

EasiVial

- Eliminates tedious weighing procedures to improve calibration accuracy
- Reduces solvent dispensing to limit risks associated with handling solvents
- For conventional and multi detector GPC to maximize applicability

For organic and aqueous GPC/SEC column calibration, this premier product is the quickest and most convenient method to deliver an accurate 12-point column calibration.

The key to achieving baseline separation from polymer mixtures, and therefore eliminating doubt and errors, is in selecting only the narrowest polydispersity polymers. This is where Agilent polymer standards excel and deliver, as shown in the chromatograms.

The EasiVial standards kit is a pre-prepared, time saving product for rapid and reliable GPC column calibration. EasiVial kits contain three vials, each with a mixture of four accurately pre-weighed polymer standards, providing a 12-point GPC calibration in just three injections. The mass of each polymer in the vial is accurately known, so that upon addition of a fixed volume of eluent, the solution is prepared at a precise concentration. EasiVial is ideal for both conventional and multi detector GPC calibration. Simply prepare and manually inject, or transfer to autosampler vials, or place directly into a compatible autosampler.

Every EasiVial kit contains 30 vials (ten of each type) that are color-coded for easy identification and are available in 4 or 2 mL vials making them suitable for most autosamplers. The kits are available for polystyrene (PS), polymethylmethacrylate (PMMA), polyethylene glycol/oxide (PEG/PEO) and polyethylene glycol (PEG). For added value, a Tri-Pack (90 vials) is offered, extending reproducibility.

EasiVial PS-H

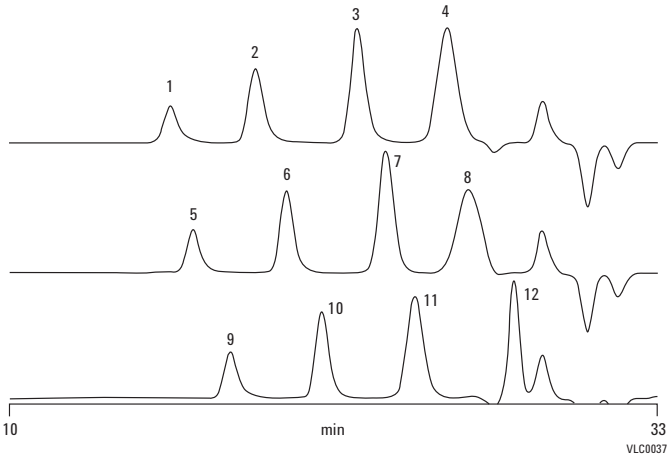
Column: **3 x PLgel 10 µm MIXED-B**
PL1110-6100
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

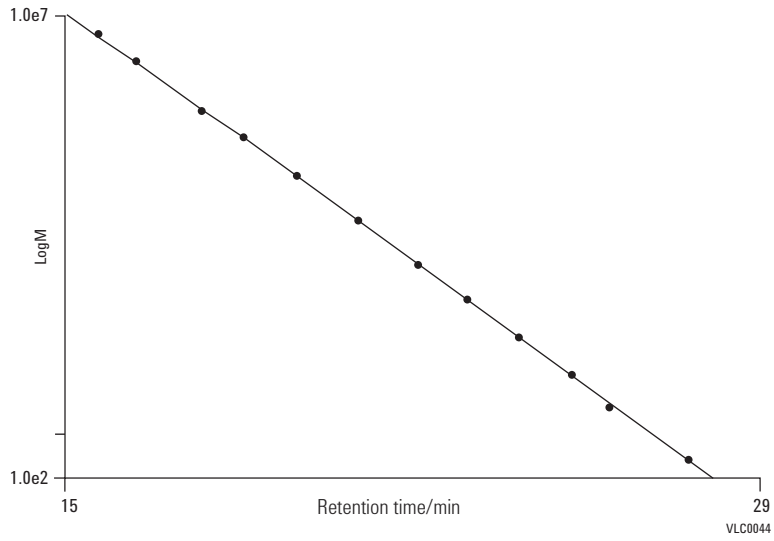
Temperature: 40 °C

Detector: PL-GPC 220 (RI)



1. 6,035,000
2. 483,000
3. 19,720
4. 1,260
5. 3,053,000
6. 84,900
7. 8,450
8. 580
9. 915,000
10. 60,450
11. 3,370
12. 162

Polystyrene calibration generated with EasiVials



Specifications

EasiVial Color	EasiVial PS-H	EasiVial PS-M	EasiVial PS-L	EasiVial PM	EasiVial PEG/PEO	EasiVial PEG
			Nominal Mp (g/mol)			
Red	1,300	780	580	2,000	600	282
	20,000	6,000	3,000	30,000	12,000	1,000
	500,000	50,000	10,000	300,000	125,000	6,000
	6,000,000	400,000	40,000	2,000,000	1,200,000	35,000
Yellow	580	370	370	1,000	200	194
	8,500	2,500	2,000	13,000	4,000	600
	185,000	25,000	6,000	150,000	60,000	3,750
	3,000,000	200,0001	25,000	800,000	1,000,000	21,000
Green	162	162	162	600	100	106
	3,400	1,500	1,000	5,700	1,500	420
	60,000	11,000	4,000	80,000	25,000	2,000
	900,000	100,000	16,000	470,000	460,000	12,000

Description Key

PS: Polystyrene
PM: Polymethylmethacrylate
PEG/PEO: Polyethylene Glycol/Oxide
H: High
M: Medium
L: Low

EasiVial Pre-weighed Calibration Kits

Description	Range of Nominal Mp (g/mol)	Vial Volume (mL)	Unit	Part No.
EasiVial PEG/PEO	100-1,200,000	2	30/pk	PL2080-0201
EasiVial PEG/PEO	100-1,200,000	4	30/pk	PL2080-0200
EasiVial PEG	106-35,000	2	30/pk	PL2070-0201
EasiVial PEG	106-35,000	4	30/pk	PL2070-0200
EasiVial PM	600-2,000,000	2	30/pk	PL2020-0201
EasiVial PM	600-2,000,000	4	30/pk	PL2020-0200
EasiVial PS-H	162-6,000,000	2	30/pk	PL2010-0201
EasiVial PS-H	162-6,000,000	4	30/pk	PL2010-0200
EasiVial PS-M	162-400,000	2	30/pk	PL2010-0301
EasiVial PS-M	162-400,000	4	30/pk	PL2010-0300
EasiVial PS-L	162-40,000	2	30/pk	PL2010-0401
EasiVial PS-L	162-40,000	4	30/pk	PL2010-0400
PEG/PEO Tri-Pack		2	90/pk	PL2080-0202
PEG/PEO Tri-Pack		4	90/pk	PL2080-0203
PEG Tri-Pack		2	90/pk	PL2070-0202
PEG Tri-Pack		4	90/pk	PL2070-0203
PMMA Tri-Pack		2	90/pk	PL2020-0202
PMMA Tri-Pack		4	90/pk	PL2020-0203
PS-H Tri-Pack		2	90/pk	PL2010-0202
PS-H Tri-Pack		4	90/pk	PL2010-0203
PS-L Tri-Pack		2	90/pk	PL2010-0402
PS-L Tri-Pack		4	90/pk	PL2010-0403

EasiCal

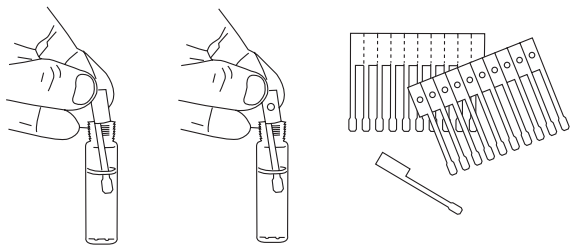
- Easy three-step process with no fuss
- Cost-effective format saves money
- Only two injections for improved productivity

The EasiCal system for organic solvents consists of two different combs, each with ten detachable spatulas, supporting a mixture of five polymer standards. The thin film of polymer (approximately 5 mg) on the tip of the PTFE spatulas rapidly dissolves when immersed in eluent to provide two GPC/SEC calibration solutions. A single pack provides ten spatulas of each type, with MWs selected to provide equidistant calibration points for greater accuracy.

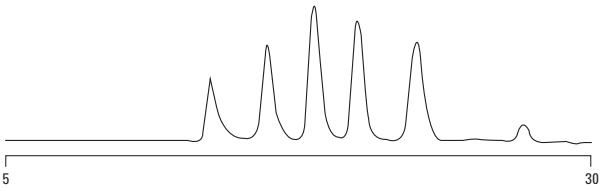
EasiCal Pre-prepared Polystyrene Kits

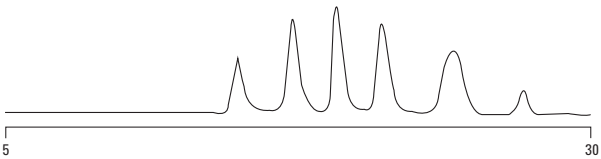
Description	Range of Nominal Mp (g/mol)	Unit	Part No.
Polystyrene PS-1	580-7,500,000	1/pk	PL2010-0501
		5/pk	PL2010-0505
Polystyrene PS-2	580-400,000	1/pk	PL2010-0601
		5/pk	PL2010-0605

Column calibration for GPC/SEC is as easy as 1, 2, 3...

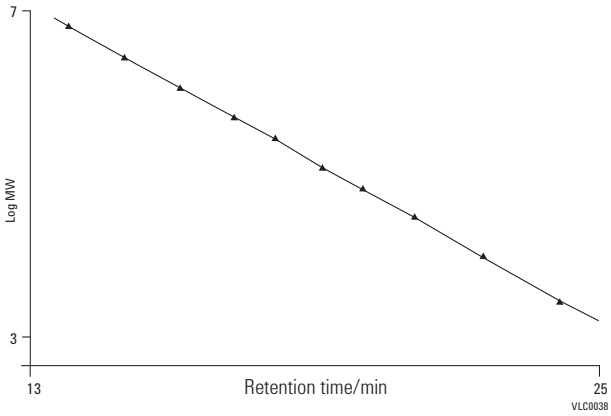


1. Place one spatula of each type into appropriate volume of solvent.





2. Chromatograph each solution; only two injections required



3. Generate a 10-point calibration

Polystyrene

- Compatible with most organic solvents
- Certificate of Analysis meets international protocols
- Calibration capability for virtually all applications

Polystyrene standards are the first choice for many organic solvents, either for conventional GPC column calibration or for calibrating light scattering and viscosity detectors. Our organic polymers cover a range from 162-15 million MW, with MWs selected to provide equidistant calibration points for greater accuracy. Every kit contains 0.5 g of ten different molecular weight standards.

Calibration Kits, (All Kits 10 x 0.5 g)

S-H-10 Part No. PL2010-0103	S-H2-10 Part No. PL2010-0104	S-M-10 Part No. PL2010-0100	S-M2-10 Part No. PL2010-0102	S-L-10 Part No. PL2010-0101	S-L2-10 Part No. PL2010-0105
Constituent Polymer Nominal Mp (g/mol)					
300,000	1,000	580	580	162	162
460,000	3,000	1,450	1,400	580	370
700,000	8,600	4,000	2,400	900	580
1,100,000	25,000	10,000	4,750	1,400	800
1,700,000	73,000	27,000	9,500	2,200	1,000
2,600,000	210,000	66,000	19,000	3,400	1,500
4,000,000	600,000	180,000	38,000	5,100	1,900
6,200,000	1,780,000	460,000	75,000	8,100	2,500
9,500,000	5,000,000	1,190,000	150,000	12,800	3,200
15,000,000	15,000,000	3,000,000	300,000	20,000	4,500

Description Key

H: High
M: Medium
L: Low

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
162	1.00	PL2012-1001	PL2012-1005	PL2012-1010
370	1.11	PL2012-0001	PL2012-0005	PL2012-0010
580	1.11	PL2012-2001	PL2012-2005	PL2012-2010
1,000	1.09	PL2012-3001	PL2012-3005	PL2012-3010
1,300	1.07	PL2012-4001	PL2012-4005	PL2012-4010
2,000	1.05	PL2012-5001	PL2012-5005	PL2012-5010
3,000	1.04	PL2012-6001	PL2012-6005	PL2012-6010
5,000	1.03	PL2012-7001	PL2012-7005	PL2012-7010
7,000	1.04	PL2012-8001	PL2012-8005	PL2012-8010
10,000	1.02	PL2012-9001	PL2012-9005	PL2012-9010
20,000	1.02	PL2013-1001	PL2013-1005	PL2013-1010
30,000	1.02	PL2013-2001	PL2013-2005	PL2013-2010
50,000	1.03	PL2013-3001	PL2013-3005	PL2013-3010
70,000	1.03	PL2013-4001	PL2013-4005	PL2013-4010
100,000	1.02	PL2013-5001	PL2013-5005	PL2013-5010
130,000	1.01	PL2013-6001	PL2013-6005	PL2013-6010
200,000	1.05	PL2013-7001	PL2013-7005	PL2013-7010
300,000	1.03	PL2013-8001	PL2013-8005	PL2013-8010
500,000	1.03	PL2013-9001	PL2013-9005	PL2013-9010
700,000	1.03	PL2014-0001	PL2014-0005	PL2014-0010
1,000,000	1.05	PL2014-1001	PL2014-1005	PL2014-1010
1,500,000	1.04	PL2014-2001	PL2014-2005	PL2014-2010
2,000,000	1.04	PL2014-3001	PL2014-3005	PL2014-3010
2,500,000	1.05	PL2014-4001	PL2014-4005	PL2014-4010
4,000,000	1.04	PL2014-6001	PL2014-6005	PL2014-6010
7,000,000	1.04	PL2014-7001	PL2014-7005	PL2014-7010
10,000,000	1.06	PL2014-8001	PL2014-8005	PL2014-8010
15,000,000	1.06	PL2014-9001	PL2014-9005	PL2014-9010

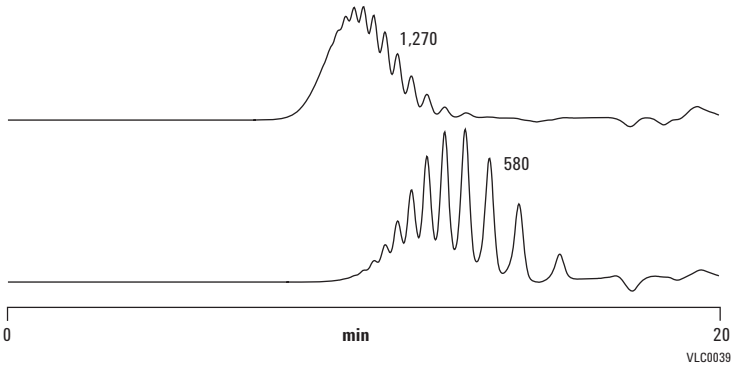
Polystyrene standards

Column: **2 x OligoPore PL1113-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Polymethylmethacrylate

- Many solvent options increase applicability
- Stringent quality control improves performance
- Proprietary manufacturing methods ensure consistent supply

Polymethylmethacrylate (PMMA) standards are extremely versatile as they can be used for organic GPC with a wide range of medium polarity eluents, such as tetrahydrofuran, toluene, methyl ethyl ketone, and ethyl acetate. They also work well with more polar organic eluents, for example dimethylformamide, dimethylacetamide, and hexafluoroisopropanol. The MWs are selected to provide equidistant calibration points for greater accuracy, covering from 500-1.5 million MW. Every kit contains 0.5 g of ten different molecular weight standards.

Calibration Kits, (All Kits 10 x 0.5 g)

M-L-10 Part No. PL2010-0100	**M-M-10 Part No. PL2020-0101**
Constituent Polymer Nominal Mp (g/mol)	
600	1,000
840	2,200
1,400	5,000
2,350	11,200
3,900	25,500
6,400	58,000
10,800	130,000
18,000	290,000
30,000	660,000
50,000	1,500,000

Description Key

M: Medium
L: Low

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
500	1.19	PL2022-2001	PL2022-2005	PL2022-2010
1,000	1.26	PL2022-3001	PL2022-3005	PL2022-3010
2,000	1.08	PL2022-5001	PL2022-5005	PL2022-5010
3,000	1.08	PL2022-6001	PL2022-6005	PL2022-6010
5,000	1.09	PL2022-7001	PL2022-7005	PL2022-7010
7,000	1.08	PL2022-8001	PL2022-8005	PL2022-8010
10,000	1.03	PL2022-9001	PL2022-9005	PL2022-9010
13,000	1.03	PL2023-0001	PL2023-0005	PL2023-0010
20,000	1.03	PL2023-1001	PL2023-1005	PL2023-1010
30,000	1.02	PL2023-2001	PL2023-2005	PL2023-2010
50,000	1.02	PL2023-3001	PL2023-3005	PL2023-3010
70,000	1.02	PL2023-4001	PL2023-4005	PL2023-4010
100,000	1.02	PL2023-5001	PL2023-5005	PL2023-5010
130,000	1.05	PL2023-6001	PL2023-6005	PL2023-6010
200,000	1.02	PL2023-7001	PL2023-7005	PL2023-7010
300,000	1.02	PL2023-8001	PL2023-8005	PL2023-8010
500,000	1.06	PL2023-9001	PL2023-9005	PL2023-9010
700,000	1.03	PL2024-0001	PL2024-0005	PL2024-0010
1,000,000	1.09	PL2024-1001	PL2024-1005	PL2024-1010
1,500,000	1.09	PL2024-2001	PL2024-2005	PL2024-2010

Polymethylmethacrylate standards

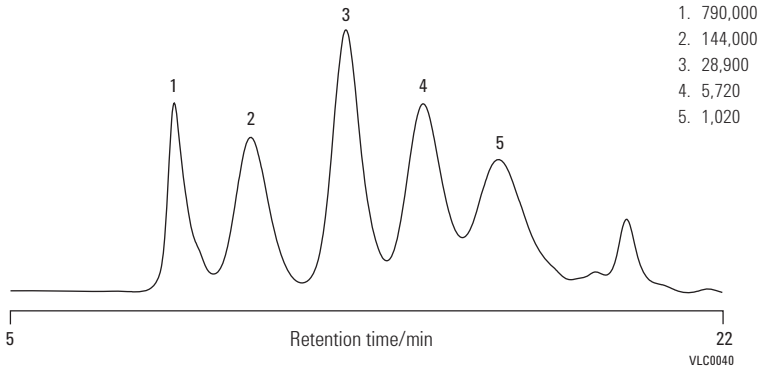
Column: **2 x PL HFIPgel PL1114-6900HFIP 7.5 x 300 mm**

Mobile Phase: HFIP + 20 mM NaTFAc

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: Agilent PL-GPC 50 (RI)



Polyethylene Glycol/Oxide

- Simple-to-use kit form
- Combines glycols and oxides to extend the MW range and cover more applications
- MWs selected to provide equidistant calibration points for greater accuracy

These hydrophilic polymers are suitable for both aqueous SEC and organic GPC using the majority of polar organic solvents. The oxides are available in high molecular weights, while the glycols cover the lower molecular weight range. The two types are chemically similar so they can be used together across a wider molecular weight range, with aqueous and organic polymers from 106-1 million MW. Every kit contains 0.2 g or 0.5 g of ten different molecular weight standards.

Calibration Kits

PEG-10 (10 x 0.5 g) **Part No.** **PL2070-0100**	**PEO-10 (10 x 0.2 g)** **Part No.** **PL2080-0101**
Constituent Polymer Nominal Mp (g/mol)	
106	20,000
194	30,000
400	50,000
600	70,000
1,000	100,000
2,000	200,000
4,000	300,000
7,000	400,000
13,000	700,000
20,000	1,000,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
Polyethylene Glycol				
106	1.00	PL2070-1001	PL2070-1005	PL2070-1010
194	1.00	PL2070-2001	PL2070-2005	PL2070-2010
238	1.00	PL2071-2001	PL2071-2005	PL2071-2010
282	1.00	PL2071-3001	PL2071-3005	PL2071-3010
420	1.09	PL2070-3001	PL2070-3005	PL2070-3010
600	1.06	PL2070-4001	PL2070-4005	PL2070-4010
1,000	1.04	PL2070-5001	PL2070-5005	PL2070-5010
1,500	1.04	PL2070-6001	PL2070-6005	PL2070-6010
4,000	1.03	PL2070-7001	PL2070-7005	PL2070-7010
7,000	1.04	PL2070-8001	PL2070-8005	PL2070-8010
10,000	1.05	PL2070-9001	PL2070-9005	PL2070-9010
13,000	1.07	PL2071-0001	PL2071-0005	PL2071-0010
20,000	1.07	PL2071-1001	PL2071-1005	PL2071-1010
Polyethylene Oxide				
20,000	1.05	PL2083-1001	PL2083-1005	PL2083-1010
30,000	1.07	PL2083-2001	PL2083-2005	PL2083-2010
50,000	1.05	PL2083-3001	PL2083-3005	PL2083-3010
70,000	1.05	PL2083-4001	PL2083-4005	PL2083-4010
100,000	1.06	PL2083-5001	PL2083-5005	PL2083-5010
130,000	1.07	PL2083-6001	PL2083-6005	PL2083-6010
200,000	1.07	PL2083-7001	PL2083-7005	PL2083-7010
300,000	1.07	PL2083-8001	PL2083-8005	PL2083-8010
500,000	1.06	PL2083-9001	PL2083-9005	PL2083-9010
700,000	1.07	PL2084-0001	PL2084-0005	PL2084-0010
1,000,000	1.12	PL2084-1001	PL2084-1005	PL2084-1010
1,500,000	1.13	PL2084-2001	PL2084-2005	PL2084-2010

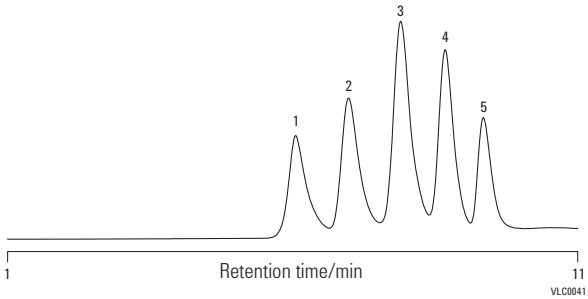
Polyethylene Glycol/Oxide standards

Column: **PL aquagel-OH MIXED-H 8 µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: Water

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



1. 1,702,000
2. 120,000
3. 12,600
4. 1,470
5. 106

Polysaccharides

- Comprehensive format provides full MW range in one handy kit
- Also available as individual standards

The pullulan polysaccharides kit consists of several simple sugars with relatively narrow polydispersity linear macromolecules of maltotriose units.

Calibration Kits

SAC-10 (10 x 0.2 g) **Part No.** **PL2090-0100**
Constituent Polymer Nominal Mp (g/mol)
180
738
5,000
10,000
20,000
50,000
100,000
200,000
400,000
700,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Unit	Part No.
1,500	0.2 g	PL2091-2000
2,000	0.2 g	PL2091-3000
3,000	0.2 g	PL2091-4000
5,000	0.5 g	PL2090-1000
20,000	0.5 g	PL2090-3000
50,000	0.5 g	PL2090-4000
100,000	0.5 g	PL2090-5000
200,000	0.5 g	PL2090-6000
700,000	0.5 g	PL2090-8000
1,660,000	0.2 g	PL2091-1000

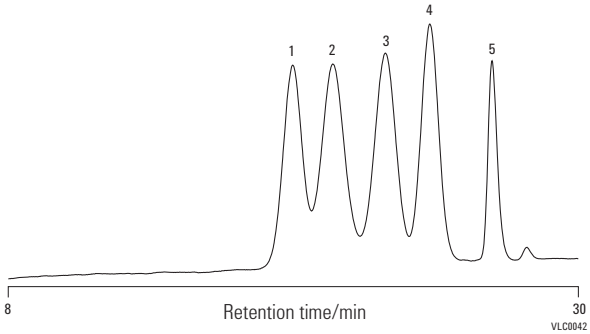
Pullulan polysaccharide standards

Column: **3 x PL aquagel-OH MIXED-H 8 µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



1. 788,000
2. 212,000
3. 47,300
4. 11,800
5. 667

Polyacrylic Acid

- Compatible with all aqueous columns for wide applicability
- Aqueous polymers 1,000-2 million MW
- Well-characterized Mp values ensure wide utility

Calibration Kits

PAA-10 (10 x 0.2 g)
Part No.
PL2140-0100

Constituent Polymer Nominal Mp (g/mol)
1,000
3,000
7,000
13,000
30,000
70,000
100,000
300,000
700,000
1,000,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	0.2 g Part No.	1 g Part No.
1,000	PL2142-3000	PL2142-3001
2,000	PL2142-5000	
3,000	PL2142-6000	PL2142-6001
5,000	PL2142-7000	PL2142-7001
7,000	PL2142-8000	PL2142-8001
13,000	PL2143-0000	PL2143-0101
30,000	PL2143-2000	PL2143-2001
50,000	PL2143-3000	PL2143-3001
70,000	PL2143-4000	PL2143-4001
100,000	PL2143-5000	PL2143-5001
130,000	PL2143-6000	PL2143-6001
200,000	PL2143-7000	PL2143-7001
300,000	PL2143-8000	PL2143-8001
500,000	PL2143-9000	PL2143-9001
700,000	PL2144-0000	PL2144-0101
1,000,000	PL2144-1000	PL2144-1001
1,500,000	PL2144-2000	PL2144-2001
2,000,000	PL2144-3000	PL2144-3001

LC and LC/MS Troubleshooting

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Baseline disturbance at void time	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
Detector leaks	Plugged inlet frit	Replace seals/gaskets
Drifting baseline	Positive direction – Contaminant buildup/elution	Flush column, clean up sample, use pure solvents
	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
	Negative direction (gradient) – Absorbance of "A" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Positive direction (gradient) – Absorbance of "B" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Random – Temperature changes	Insulate column and tubing
	Random – Temperature changes	Thermostat column and tubing
	Wavy or undulating – Temperature changes in room	Monitor room temperature and control
Ghost peaks	Peaks from previous injection	Flush column to remove contaminants
	Contamination	Sample cleanup or pre-fractionation
	Unknown interferences in samples	Sample cleanup or pre-fractionation
	Ion-pair – Upset equilibrium	Prepare sample in actual mobile phase to minimize disturbance
	Peptide mapping – Oxidation of TFA	Prepare fresh daily; use anti-oxidant
	Reversed-phase – Contaminated water	Check suitability of water by running different amount through reversed-phase column and measure peak height with elution; use HPLC grade solvents
	Spikes – Bubbles in solvent	De-gas solvents
High column backpressure	Column blockage, adsorbed sample	Better sample cleanup; use guard column
	Mobile phase viscosity too high	Use lower viscosity solvents or higher temperature
	Particle size too small	Use larger d_p packing
	Plugged inlet frit	Replace column
	Plugged inlet frit	Reverse solvent flow
Leaks	Subtle – White powder at fitting/loose fitting	Tighten fittings, cut tubing, or replace ferrules
Leaks, injection valve	Catastrophic – Worn valve rotor	Replace rotor in valve
Leaks, column or other fittings	Catastrophic – Loose fittings	Tighten or replace fittings
Leak, pump	Catastrophic – Pump seal failure	Replace pump seal

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Negative peaks	RI detector – solute refractive index less than solvent	No problem; reverse polarity to make positive
	UV detector – solute absorbance less than mobile phase	Use mobile phase with lower UV absorbance; do not recycle solvent too long
Noisy baseline	Random – Contaminant buildup	Flush column; clean up sample; use HPLC-grade solvent
	Continuous – Detector lamp problem	Replace detector lamp
	Occasional – External electrical interference	Use voltage stabilizer for LC system
Peak doubling	Sample volume too large	Reduce the volume e.g. by half and re-inject
	Injection solvent too strong	Use weaker injection solvent or mobile phase
	Blocked frit	Replace and use 0.5 µm porosity in-line filter
	Column void or channeling	Replace column; for some columns, fill in void with packing
	Unswept injector flowpath	Replace injector rotor
	Void at head of column	Replace column, top off column with packing
	Column overloaded with sample	Use higher capacity stationary phase Increase column diameter Decrease sample size
	Single peak – interfering components	Sample cleanup; pre-fractionation
Peak tailing	Beginning of peak doubling	See "peak doubling"
	Unswept dead volumes	Minimize number of connections Ensure injector seal is tight Ensure fittings are properly seated
	Basic compounds – Silanol interactions	Choose endcapped bonded phase Switch to polymeric phase
	Basic substances – Silanol interactions	Use stronger mobile phase or add competing base (e.g. TMA)
	Silica-based – Column degradation	Use specialty column; polymeric column or sterically protected

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Peaks are broad	Injection volume too large	Decrease solvent strength of injection solvent to focus solute
	Peak dispersion in injector valve	Introduce air bubble in front/back of sample to decrease dispersion
	Sampling rate of data system too slow	Increase frequency of sampling
	Slow detector time constant	Adjust time constant to match peak width
	Mobile phase viscosity too high	Increase column temperature
	Detector cell volume too large	Use smallest possible cell volume with no heat exchanger in system
	Injector volume too large	Decrease injection volume
	Long retention times	Use gradient elution or stronger mobile phase
Pressure fluctuation	Leaky check valve	Replace check valve
	Pump seal leaks	Replace pump seals
	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
Pressure increasing	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
	Water/organic systems – buffer precipitation	Test buffer-organic mixtures; ensure compatibility
Retention beyond total permeation volume	Size exclusion – Specific interactions	Add mobile phase modifiers or change solvent
Retention times changing	Column temperature varying	Thermostat column; insulate column; ensure lab temperature constant
	Equilibration time insufficient with gradient run or changes in isocratic mobile phase	Make sure at least 10 column volumes pass through column after solvent change or gradient conclusion
	Selective evaporation of mobile phase component	Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase
	Buffer capacity insufficient	Use >20 mM concentration of buffer
	Inconsistent on-line mobile phase mixing	Ensure gradient system delivering constant composition; check vs. manual prep of mobile phase
	Contamination buildup	Occasionally flush column with strong solvent to remove contaminants
	First few injections – Adsorption on active sites	Condition column by initial injection of concentrated sample

(Continued)

HPLC Troubleshooting		
Symptom Type	**Possible Cause**	**Solution**
Retention times decreasing	Flow rate increasing	Check pump to make sure correct; if not, reset
	Column overloaded with sample	Decrease sample size
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
Retention times increasing	Flow rate is slowing	Fix leaks in liquid lines, replace pump seals, check for pump cavitation or air bubbles
	Active sites on silica packing	Use mobile phase modifier
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
	Mobile phase composition changing	Make sure mobile phase container is covered
	Active sites on silica packing	Add competing base to mobile phase
	Active sites on silica packing	Use higher coverage packing for stationary phase
Sensitivity problem	Peaks are outside of linear range of detector	Dilute/concentrate to bring into linear region
	First few sample injections – Absorption of sample in loop or column	Condition loop/column with concentrated sample
	Autosampler flow lines blocked	Check flow and make sure there are no blockages
	Injector sample loop underfilled	Make sure that loop is overfilled with sample
	Sample-related losses during preparation	Use internal standard during sample prep; optimize sample prep method
Slow column equilibration times (ion-pairing)	Equilibration time slow for long-chain ion-pairing reagents	Use shorter alkyl chain ion-pair reagent

LC/MS Troubleshooting

Symptom Type	Solution
No peaks	Spray from the nebulizer
	Make sure capillary voltage is set correctly
	Make sure LC/MSD is tuned correctly
	Make sure LC/MSD pressures are within normal ranges
	Check drying gas flow and temperature
	Make sure fragmentor is set correctly
Poor mass accuracy	Recalibrate the mass axis
	Make sure ions used for tuning span mass range of sample ions and show strong stable signals
Low signal	Check the solution chemistry; make sure solvent is appropriate for sample
	Make sure sample is fresh and has been stored correctly
	Make sure LC/MSD is tuned correctly
	Check the nebulizer condition
	Clean the capillary entrance
	Check the capillary for damage and contamination
Unstable signal	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow

(Continued)

LC/MS Troubleshooting

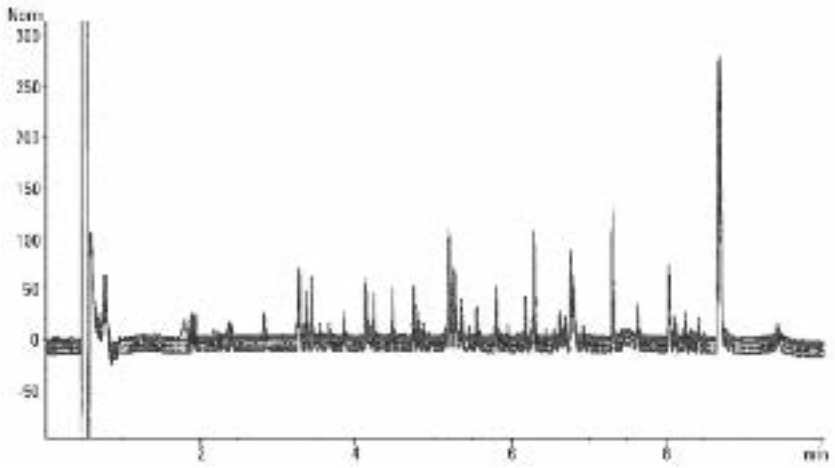
Symptom Type	Solution
High spectral noise	Use appropriate mass filter values
	Check spray shape; nebulizer may be damaged or set incorrectly
	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow
	If you are using water as part of the mobile phase, make sure it is de-ionized (> 18 MΩ cm)
Droplets, not spray, exiting the nebulizer	Make sure nebulizing gas pressure is set high enough for the LC flow
	Check position of needle in nebulizer
	Stop solvent flow and remove nebulizer assembly
	Examine end of nebulizer for damage
No flow	Make sure LC is on and there is sufficient solvent in correct bottle
	Check for LC error messages
	Check for blockages
	Repair or replace any blocked components
	Check for leaks
	Make sure MS stream selector valve is set to LC to MSD
Undesired fragmentation	(APCI vs. Electrospray)
	APCI temperature is too high
	Fragmentor voltage is set too high

BioPharmaceutical Applications

NEW!

Protein digest analysis

Column:	**ZORBAX 300SB-C18 858750-902 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.085% TFA in ACN
Flow Rate:	0.5 mL/min
Pressure:	640 bar
Gradient:	2% B 1 min, 2-45% B 8.8 min, 45-95% B 0.2 min, 95% B 2 min, 98-2% B 0.2 min, 2% B 1.8 min
Temperature:	50 °C
Detector:	Agilent 1290 Infinity LC
Injection:	5 µL
Sample:	Protein digest
Sample Conc:	1 mg/mL

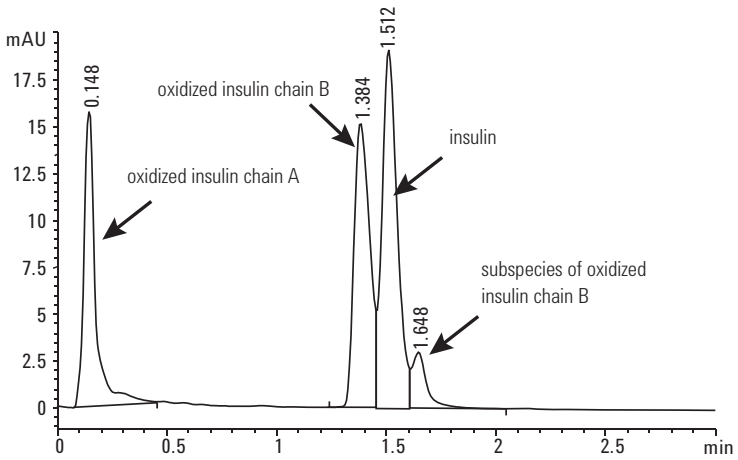


Overlaid chromatograms of 30 runs of a protein digest on an Agilent ZORBAX RRHD 300SB-C18 column.

NEW!

Analysis of oxidized insulin chains

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% ACN + 0.01% TFA in water
Flow Rate:	1.0 mL/min
Pressure:	650-700 bar
Gradient:	33-50% B, 0-4 min; 33% B, 4-5 min
Detector:	UV, 280 nm Agilent 1290 Infinity LC
Sample:	Insulin, oxidized insulin chain A and chain B from bovine pancreas (Sigma Aldrich, St. Louis, MO)
Sample Conc:	1 mg/mL
Injection:	2 µL

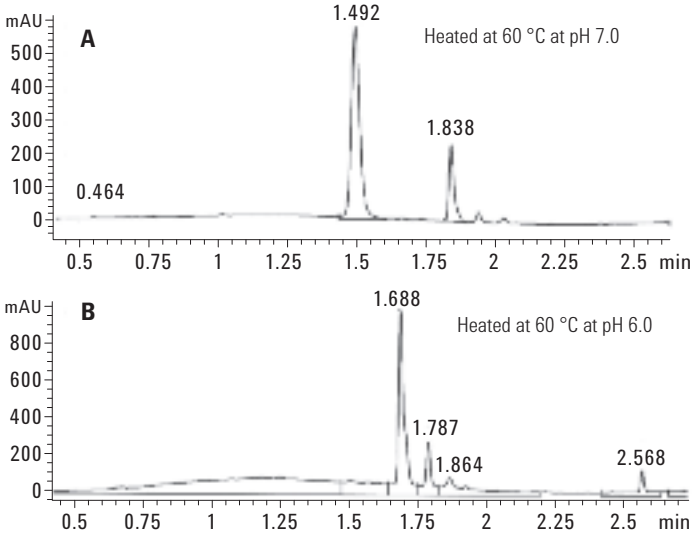


Insulin and oxidized insulin A and B chains are resolved quickly but insulin and oxidized chain B often co-elute.

NEW!

Fast separation of recombinant human erythropoietin

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.01% TFA in ACN
Flow Rate:	1.0 mL/min
Pressure:	650 bar
Gradient:	5 to 100% B solvent from 0 to 2.5 min
Detector:	UV, 280 nm Agilent 1290 Infinty LC
Sample:	Recombinant human EPO protein (rEPO)
Sample Conc:	1.0 mg/mL
Injection:	3 µL



Heat-treated rEPO protein are well resolved by the Agilent ZORBAX RRHD 300SB-C18 column. The column separated these heat-treated rEPO proteins.

NEW!

Separation optimization for ultra fast analysis of reduced and alkylated monoclonal antibody

Column: **ZORBAX RRHD 300SB-C8**
858750-906
2.1 x 100 mm, 1.8 µm

Mobile Phase: (Various)
A: H_2O + 0.1% TFA (v/v)
B: n-propanol:ACN:H_2O (80:10:10) + 0.1% TFA (v/v)

Injection: 1-3 µL

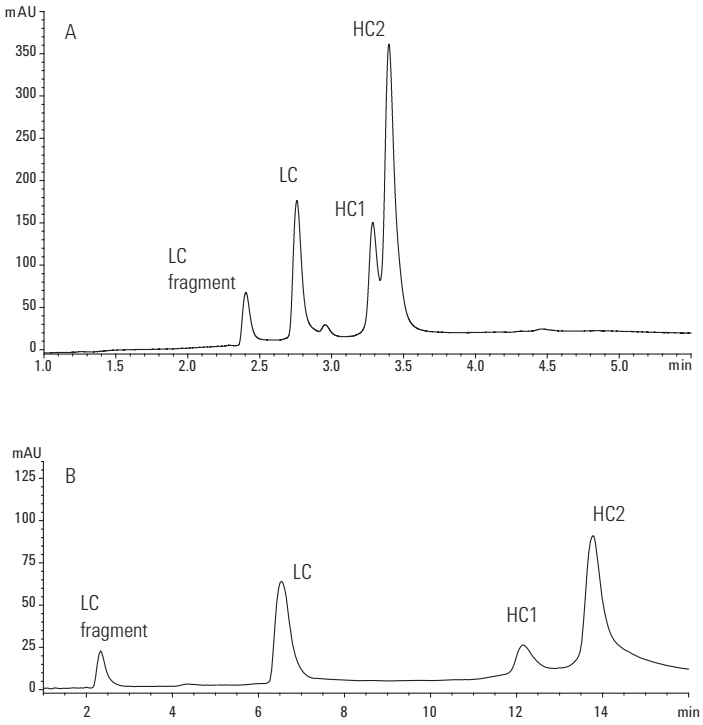
Flow Rate: 0.5 mL/min

Gradient: Multi-segmented
A (optimized for speed): 0 min-20% B, 3 min-35% B, 4 min-40% B, 5 min-40% B, 5.1 min-90% B, 5.5 min-90% B, 6 min-25% B
B (optimized for resolution): 0 min-25% B, 15 min-32% B, 16 min-32% B, 17 min-90% B, 17.5 min-90% B, 18 min-25% B

Temperature: 75 °C

Detector: UV, 225 nm
Agilent 1290 Infinity LC

For consecutive chromatographic runs, a 2-minute post run was added to re-equilibrate the column.



Comparison of two optmimized gradients for the ultra fast separation of reduced and alkylated monoclonal antibodies on an Agilent ZORBAX RRHD 300SB-C8 column. The top panel details a rapid separation of the light and heavy chain variants in a shortened run time of less than 4 minutes. The bottom panel displays complete baseline resolution of the two heavy chain variants during a longer runtime using a shallower gradient profile. Both separations were performed at 75 °C and completed with a fast 90% 1-propanol wash step (UV not shown).

NEW!

Column reproducibility – 200 injections of reduced monoclonal antibody using an Agilent ZORBAX RRHD 300SB-C3 column

Column: **Agilent ZORBAX RRHD 300SB-C3**
858750-909
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA in water
B: 80% n-propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA

Flow Rate: 0.4 mL/min

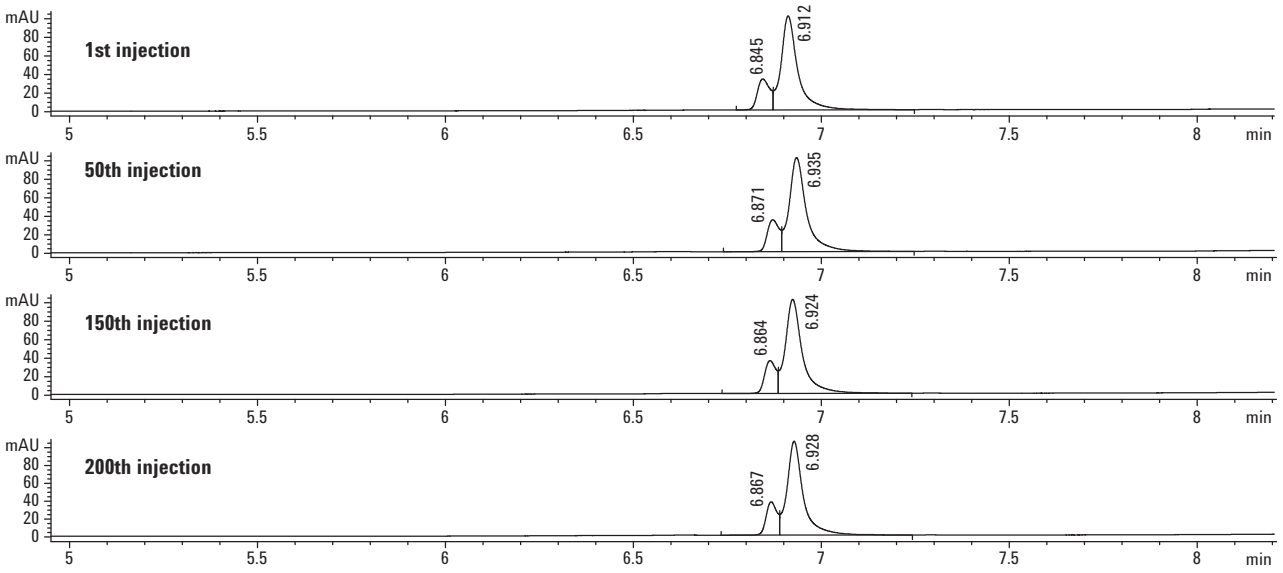
Gradient: 0 min-1% B, 2 min-20% B, 5 min-50% B, 7 min-50% B, 8.0 min-90% B, 8.3 min-1% hold for 2 min

Temperature: 75 °C

Detector: UV, 280
Agilent 1290 Infinity LC

Sample: Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - Agilent BL05 IgG1

Injection: 2 µL



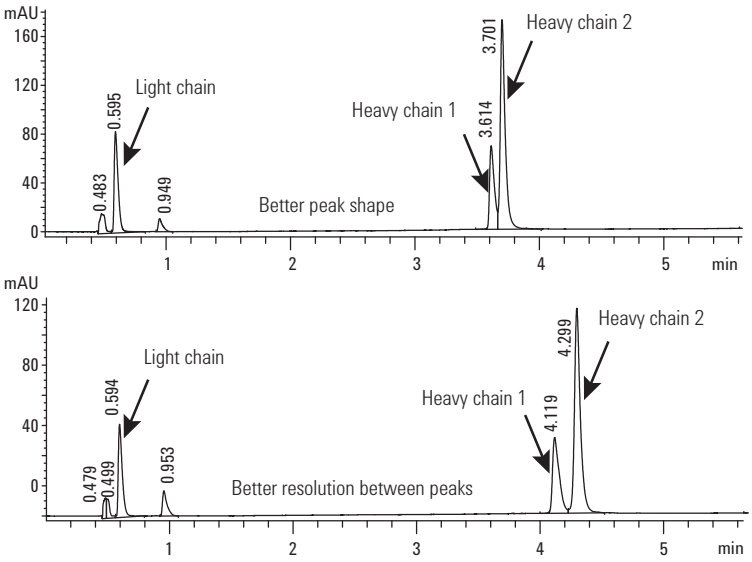
Reduced and alkylated mAb profiling during 200 repeated injections.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Gradient optimizations for ultra fast analysis of reduced monoclonal antibody

Column:	**Agilent ZORBAX RRHD 300SB-Diphenyl 858750-944 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA
Flow Rate:	0.5 mL/min
Gradient:	1st condition: 0 min-1% B, 2 min-20% B, 5 min-70% B 2nd condition: 0 min-1% B, 2 min-20% B, 5 min-50% B
Temperature:	74 °C
Detector:	UV, 280 nm
Sample:	Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1
Injection:	2 µL



Comparison of two ultra-fast separations of reduced monoclonal antibodies was achieved on a Agilent ZORBAX RRHD 300SB-Diphenyl under different optimized conditions. The top panel separation delivered narrow peak widths with shorter retention times. The bottom panel separation displays higher resolution between the two heavy chain peaks, but with less efficiency.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Ultra high speed and high resolution of intact monoclonal antibodies

Column: **Agilent ZORBAX RRHD 300-Diphenyl**
858750-944
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA in water
B: 80% n-propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA

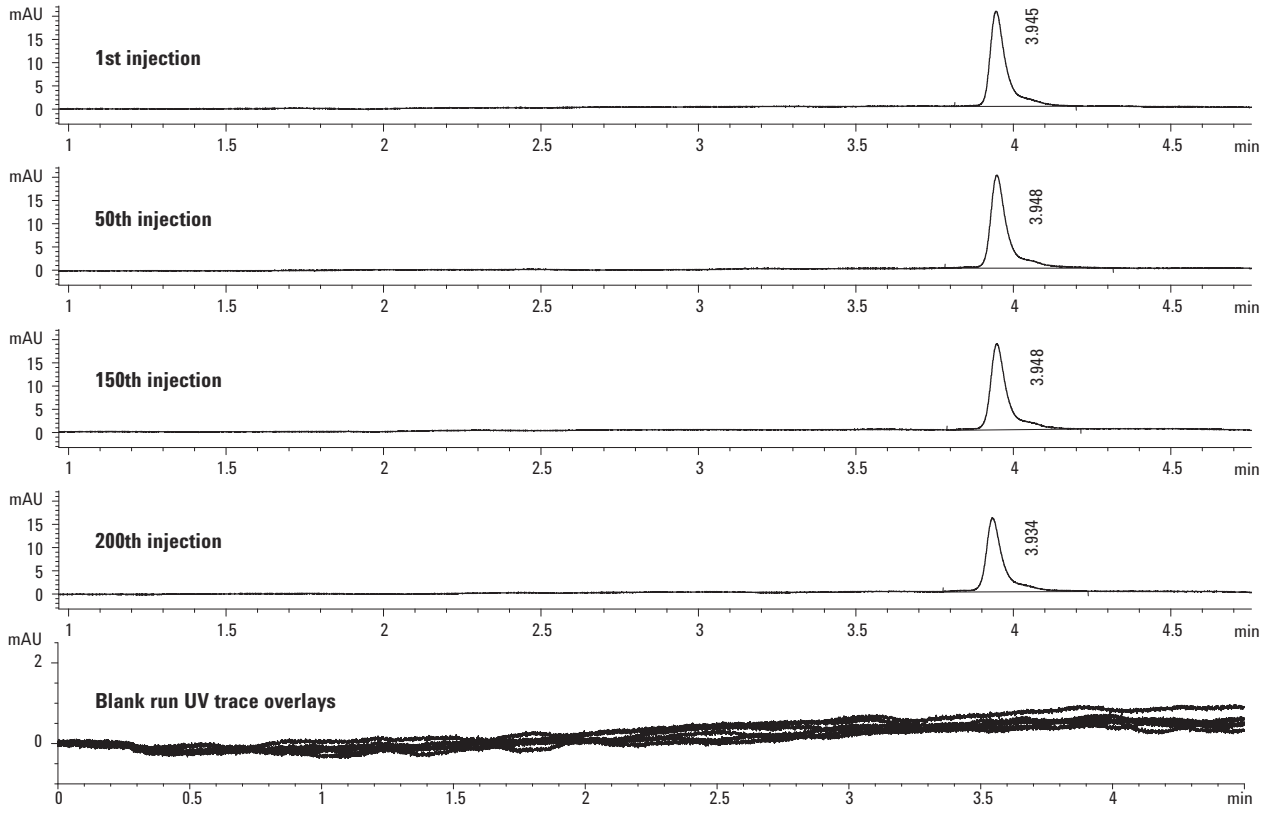
Flow Rate: 1.0 mL/min

Temperature: 74 °C

Detector: UV, 280 nm

Sample: Monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1 and Agilent Standard IgG1

Injection: 1 µL



Details of intact mAb profiling during 200 repeated injections. Intact mAb separations shown were collected at 1, 50, 150, and 200th run intervals. The bottom panel displays 5 UV blank run trace overlays collected every 20th run during the column evaluation (**note:** overlay traces are scaled to 2 mAu).

NEW!

Optimizing protein separations with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 40.0 mM NaH_2PO_4
D: 40.0 mM Na_2HPO_4
By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)

Flow Rate: 1.0 mL/min

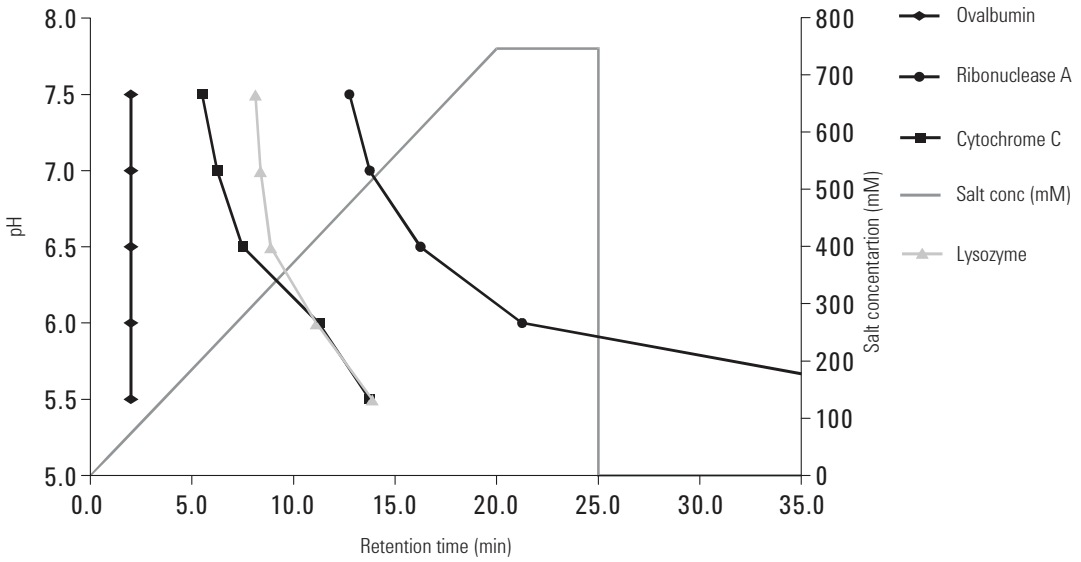
Gradient: 0 to 50% B, 0 to 20 min
50% B, 20 to 25 min
0% B, 25 to 35 min

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Effect of pH on retention time of protein standards using a Agilent Bio WCX column.

NEW!

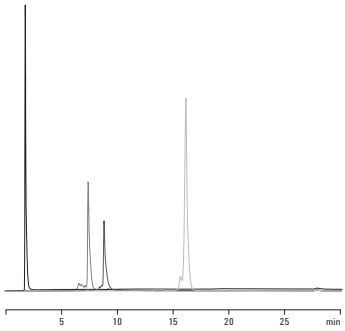
Improved resolution with smaller particle size with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

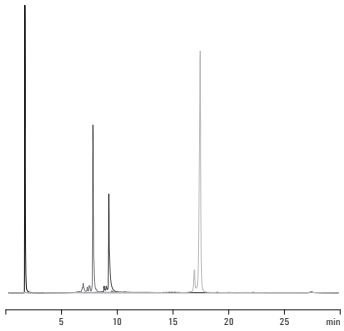
Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase:	A: water B: 1.6 M NaCl C: 40.0 mM NaH_2PO_4 D: 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)
Gradient:	0 to 50% B, 0 to 20 min 50% B, 20 to 25 min 0% B, 25 to 35 min
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme



Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP10 column.

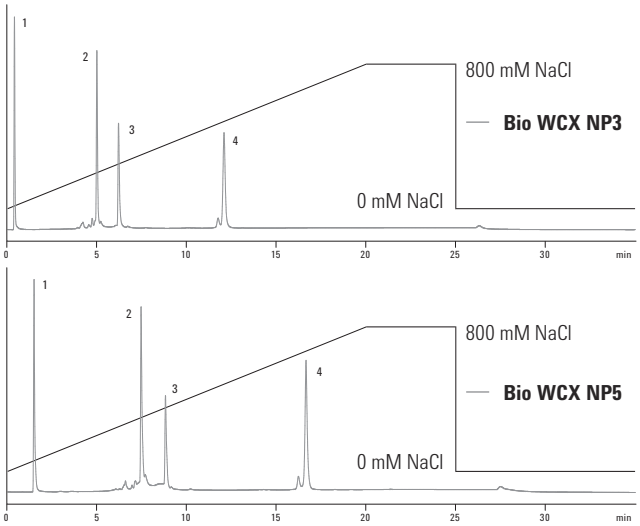


Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP5 column.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

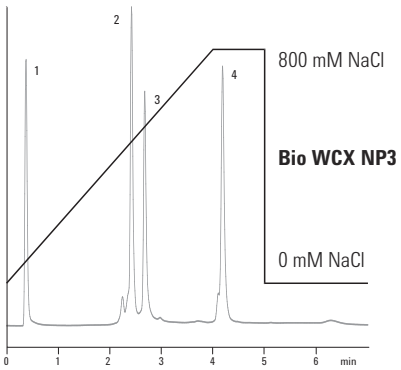
Faster separations using Agilent weak cation-exchange columns

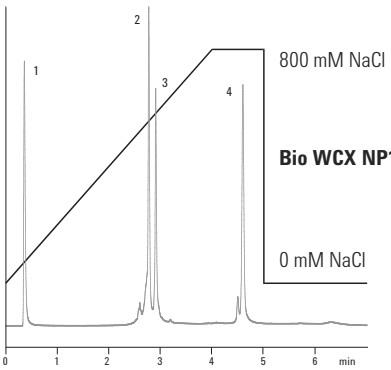


Protein separation on Agilent Bio WCX NP5 versus Agilent Bio WCX NP3.

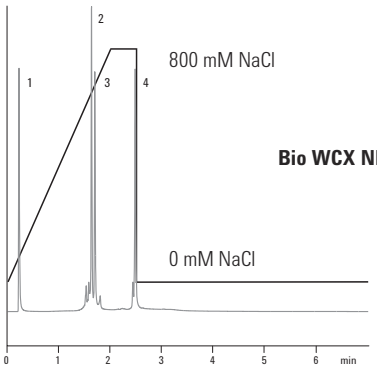
Column:	**Agilent Bio WCX, stainless steel 5190-2445 4.6 x 250 mm, 5 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2443 4.6 x 50 mm, 3 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2441 4.6 x 50 mm, 1.7 µm**
Mobile Phase:	A: 20 mM sodium phosphate, pH 6.5 B: A + 1.6 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	0 to 50% B
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	0.5 mg/mL

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme





Comparison of Agilent Bio WCX NP3 versus Agilent Bio WCX NP1.7 (flow rate 1.0 mL/min).



Agilent Bio WCX NP1.7 for protein separations under 3 minutes (flow rate 1.7 mL/min).

NEW!

pH gradient elution for improved separation of monoclonal antibody charged variants

Column: **Bio MAb, stainless steel**
5190-2405
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 100 mM NaH_2PO_4
D: 100 mM Na_2HPO_4
By combining predetermined proportions of C and D, buffer solutions at the desired pH range were produced at the selected buffer strengths.

Flow Rate: 1.0 mL/min

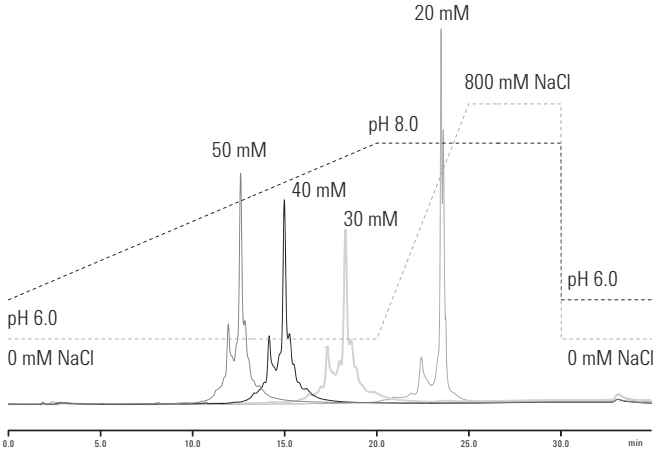
Gradient: pH 6.0 to 8.0, 0 to 20 minutes
0 to 800 mM NaCl, 20 to 25 minutes
800 mM NaCl, 25 to 30 minutes

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: IgG monoclonal antibody

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Chromatograms of IgG monoclonal antibody at different ionic strengths.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Separation of recombinant human erythropoietin (rEPO) using Agilent Bio SEC-3

Column: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

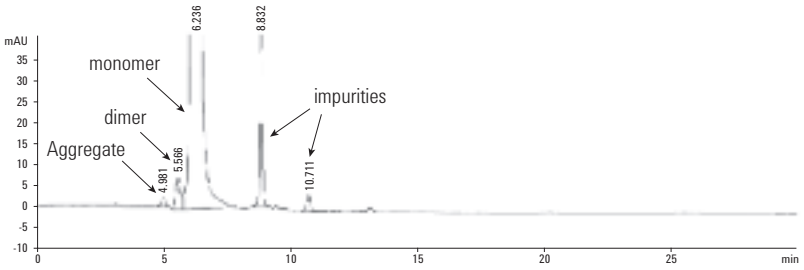
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0

Flow Rate: 0.35 mL/min

Detector: UV, 225 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Recombinant human EPO protein (rEPO)

Sample Conc: 1.0 mg/mL



Consistent ion-exchange MAb separation

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

Buffer: A: Sodium phosphate buffer, 20 mM
B: Buffer A + 400 mM NaCl

Gradient: 15-35% Buffer B from 0-30 min

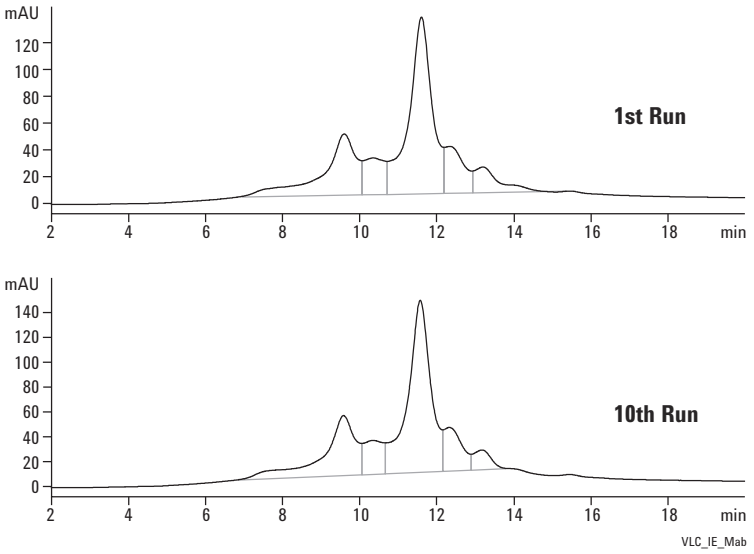
Flow Rate: 0.65 mL/min

Sample: CHO-humanized MAb, 1 mg/mL

Injection: 2.5 µL

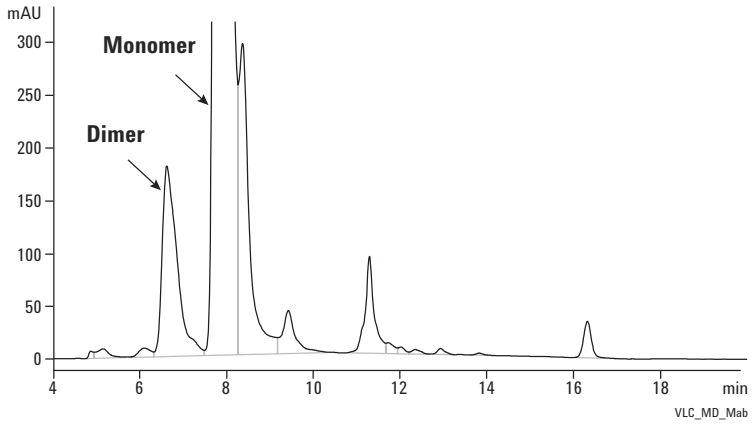
Detector: UV, 220 nm

Temperature: Ambient



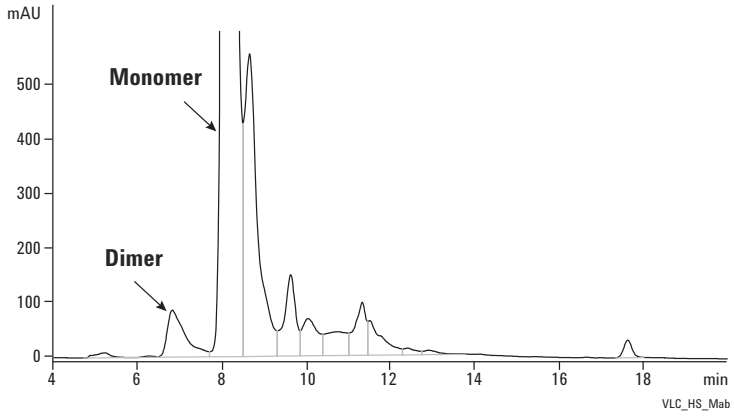
Intact MAb monomer and dimer separation

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM
Gradient:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – intact
Injection:	5 µL
Detector:	UV, 220 nm
Temperature:	Ambient



Separation of heated, stressed MAb

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM +150 mM sodium sulfate
Gradient:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – stressed at 60 °C
Injection:	5 µL
Detector:	UV, 220 nm
Temperature:	Ambient



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Nucleosides, purines and pyrimidines

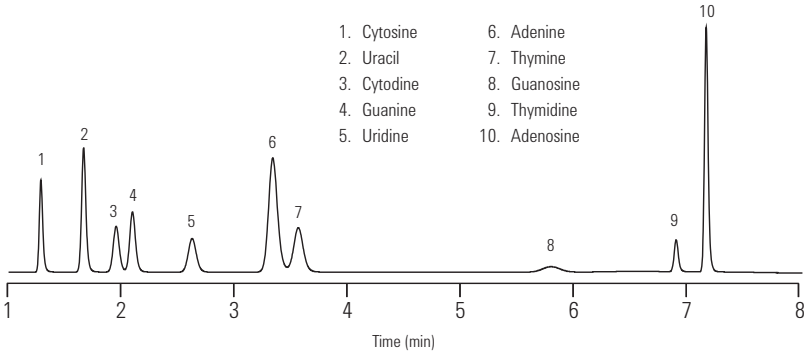
Column: **Eclipse Plus Phenyl Hexyl**
959993-912
4.6 x 150 mm, 5 µm

Mobile Phase: 1% MeOH: 99% 20 mm Ammonium Acetate, pH 4.5

Flow Rate: 1 mL/min

Detector: UV, 254 nm

1. Cytosine
2. Uracil
3. Cytodine
4. Guanine
5. Uridine
6. Adenine
7. Thymine
8. Guanosine
9. Thymidine
10. Adenosine



nucleosides

Amino acid standard separation on Eclipse Plus C18

Column: **Eclipse Plus C18**
959763-902
2.1 x 150 mm, 3.5 µm

Mobile Phase: A: 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3, pH 8.2
B: acetonitrile: methanol: water (45:45:10) (v/v/v)

Flow Rate: 0.42 mL/min

Temperature: 40 °C

Detector: UV, 338 nm, then switch to 280 nm at 15.7 min

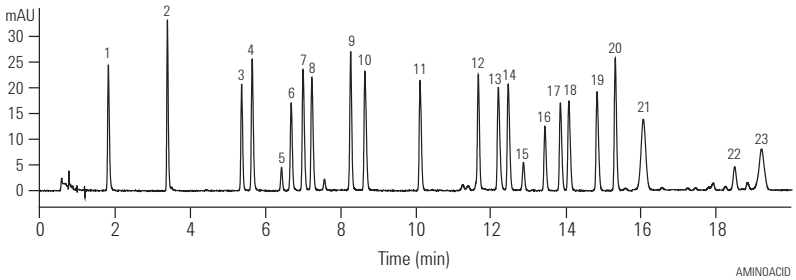
Sample: 900 pmol Amino Acids with extended Amino Acids and Internal Standards (500 pmol)

Derivatization: Automated, online, OPA / FMOC

1. ASP
2. GLU
3. ASN
4. SER
5. GLN
6. HIS
7. GLY
8. THR
9. ARG
10. ALA
11. TYR
12. CY2
13. VAL
14. MET
15. NVA
16. TRP
17. PHE
18. ILE
19. LEU
20. LYS
21. HYP
22. SAR
23. PRO

Gradient

Time (min)	% B
0	2
0.5	2
20	57
20.1	100
23.5	100
23.6	2
25	stop



AMINOACID

Antibodies: Fast separation of IgM and IgG antibodies

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

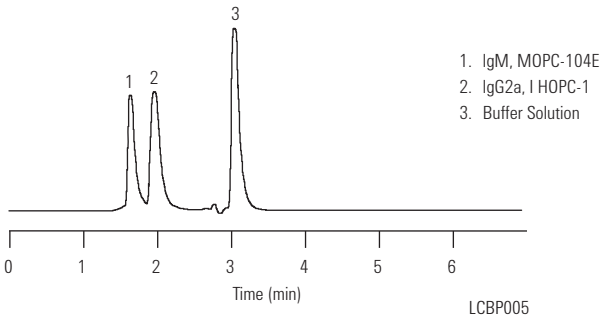
Mobile Phase: 200 mM Sodium Phosphate (pH 7), 0.01% Azide

Flow Rate: 0.94 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 2.5 µL (1 mg/mL)



Glycosylated proteins: Large molecules on Poroshell 300SB-C18 and 300SB-C8

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

Column B: **Poroshell 300SB-C8**
661750-906
1.0 x 75 mm, 5 µm

Column C: **ZORBAX 300SB-C18**
865630-902
1.0 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.07% TFA in ACN

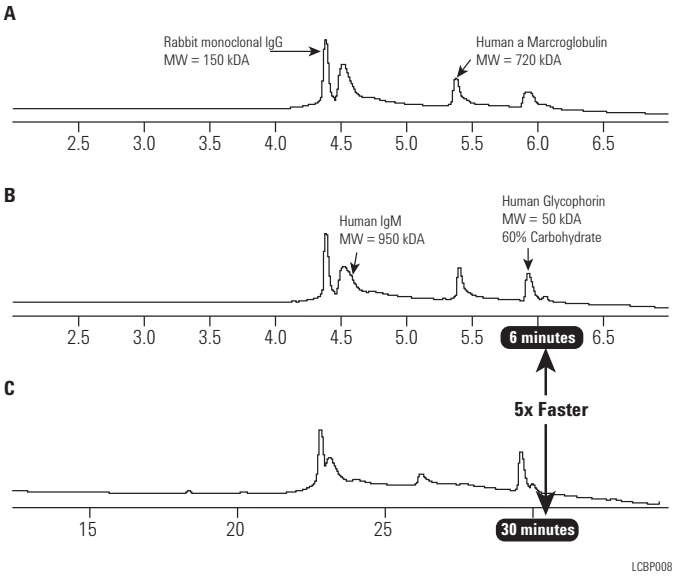
Flow Rate: A, B: 0.454 mL/min
C: 0.071 mL/min

Gradient: A, B: 0 min 5% B
10 min 100% B
C: 0 min 5% B
50 min 100% B

Temperature: 70 °C

Detector: DAD 212 nm, 1.7 µL flow cell, <0.01 min peak width

Sample: Large glycosylated proteins



Courtesy of:
Novartis AG, Basel.
Dr. Kurt Forrer
Patrik Roethlisberger

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

HSA tryptic digest on ZORBAX Rapid Resolution HT 1.8 µm

Column A: **ZORBAX SB-C18**
883700-922
2.1 x 150 mm, 5 µm

Column B: **ZORBAX SB-C18**
822700-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: Water w/0.1% TFA
B: ACN w/0.1% TFA

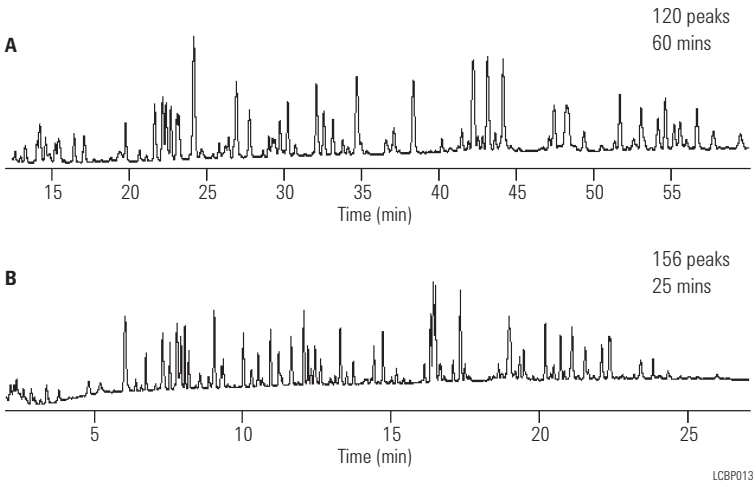
Flow Rate: A: 0.2 mL/min
B: 0.5mL/min

Gradient: A: 2 to 50% B in 70min
B: 2 to 50% B in 30min

Temperature: 50 °C

Detector: UV, 214 nm

Sample: HSA tryptic digest, 8 µL of 15 pmol/µL (120 pmol on column)



Human serum: Low abundance protein isolation and identification from 1-D gel band by LC/MS

Column: **ZORBAX 300SB-C18**
Trap: 0.3 x 5 mm, 5 µm, 5065-9913
Analytical: 0.3 x 150 mm,
5 µm, 5064-8263

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

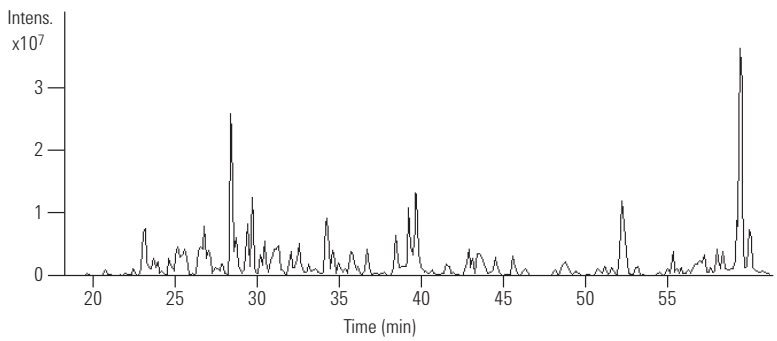
Flow Rate: 6 µL/min

Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

Sample: Band from 1-D in gel digest

Sample Preparation of Human Serum:
Major serum proteins removed using Multiple Affinity Removal
Column: 4.6 x 100 mm, P/N 5185-5985
Followed by 1-D gel digest

Base Peak Chromatogram



Proteins Identified

1. α-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

Monoclonal IgG1 chains: Separation on Poroshell 300SB-C8

Column: **Poroshell 300SB-C8**
660750-906
2.1 x 75 mm, 5 µm

Mobile Phase: A: 90% water: 10% ACN + 3 mL/L of MW 300 PEG
B: 10% water: 90% ACN + 3 mL/L of MW 300 PEG

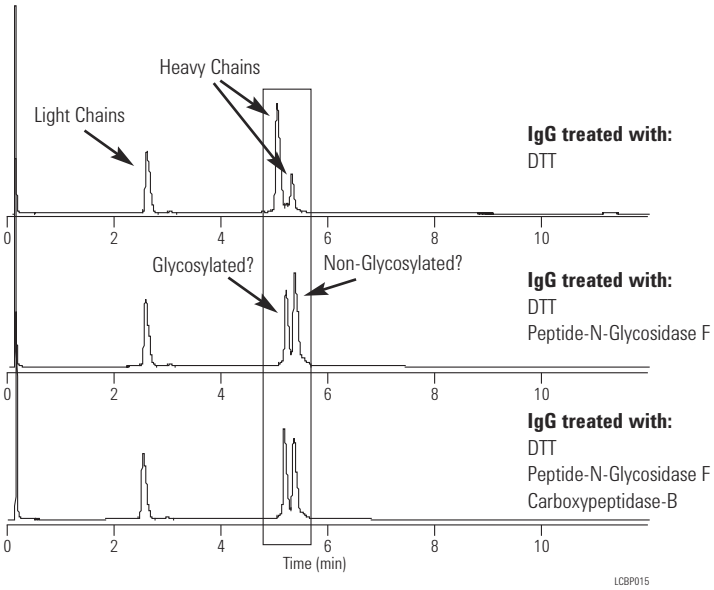
Flow Rate: 1.0 mL/min

Gradient: 0 min 25% B
10 min 40% B
10.1 min 25% B
12 min 25% B

Temperature: 70 °C

Sample: Monoclonal IgG1

Courtesy of:
Novartis AG, Basel.
Dr. Kurt Forrer
Patrik Roethlisberger



Use ZORBAX Extend-C18 for alternate selectivity at high pH

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

Mobile Phase: A: 0.1% TFA in Water
B: 0.085% TFA in 80% ACN

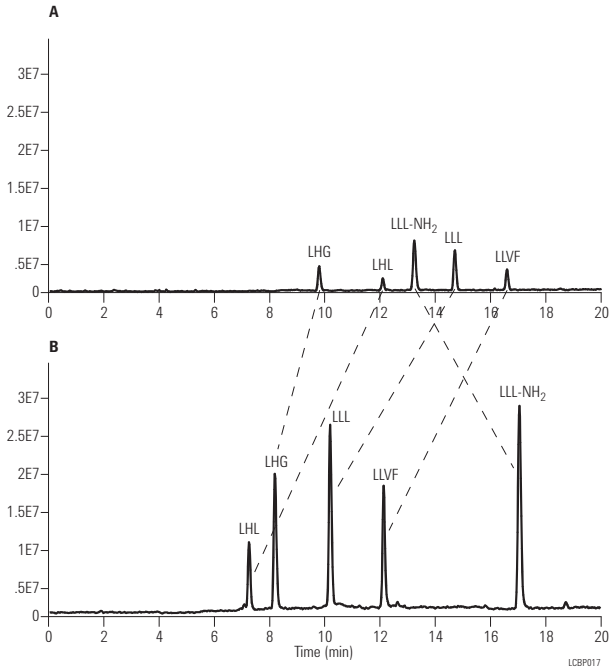
A: 20 mM NH_4OH in Water
B: 20 mM NH_4OH in 80% ACN

Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV
N_2 – 35 psi, 12 L/min, 300 °C
4 µL (50 ng each peptide)



The Extend column can be used for high pH separations of peptides. At high and low pH, very different selectivity can result. Just by changing pH, a complementary method can be developed and it is possible to determine if all peaks are resolved. The Extend column can be used at high and low pH, so the complementary separation can be investigated with one column. Better MS sensitivity for this sample is also achieved at high pH.

Nucleosides: Separation of deoxy and ribonucleosides

Column: **ZORBAX SB-C3**
883975-909
4.6 x 150 mm, 5 µm

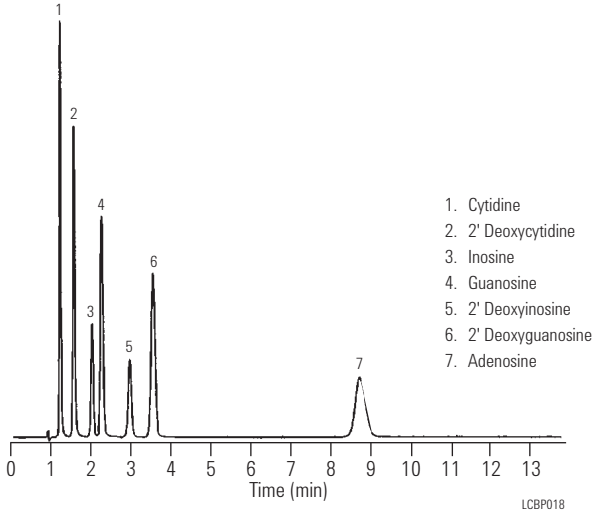
Mobile Phase: 4 mM Ammonium Phosphate (pH 4.0 with Phosphoric Acid)

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL (1.6 µg each)



1. Cytidine
2. 2' Deoxycytidine
3. Inosine
4. Guanosine
5. 2' Deoxyinosine
6. 2' Deoxyguanosine
7. Adenosine

Nucleotides: Separation of mononucleotides

Column: **ZORBAX SAX**
880952-703
4.6 x 250 mm, 5 µm

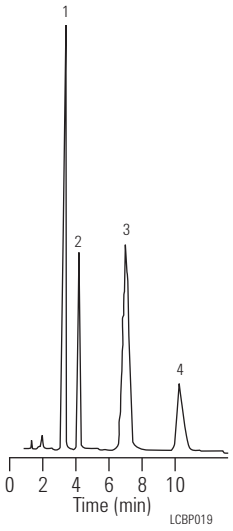
Mobile Phase: 0.1 M $NH_4H_2PO_4$

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Orotic Acid, UMP, GMP, XMP



1. Orotic Acid
2. UMP
3. GMP
4. XMP

Separation of basic peptides on Bonus-RP versus traditional Alkyl phase

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

Column B: **Alkyl C8**

Mobile Phase: A: 0.010 M ammonium phosphate, pH 7/0.050 M sodium perchlorate
B: 0.010 M ammonium phosphate/0.050 M sodium perchlorate in 50% ACN

Flow Rate: 1.0 mL/min

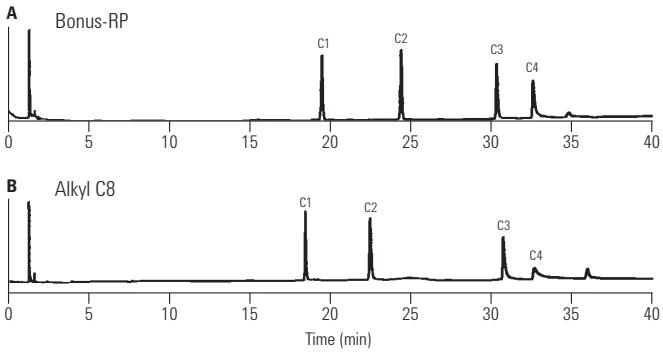
Gradient: 0-100% B in 50 min

Temperature: 40 °C

Detector: 215 nm

Sample: Basic 11-residue peptides with net +1, +2, +3, +4 positive charges at neutral pH

C1: Ac-Gly-Gly-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide
C2: Ac-Lys-Tyr-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide
C3: Ac-Gly-Gly-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide
C4: Ac-Lys-Tyr-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide



LCBP020

Peptides: Effect of TFA concentration

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

Mobile Phase: A: Water and TFA
B: ACN and TFA

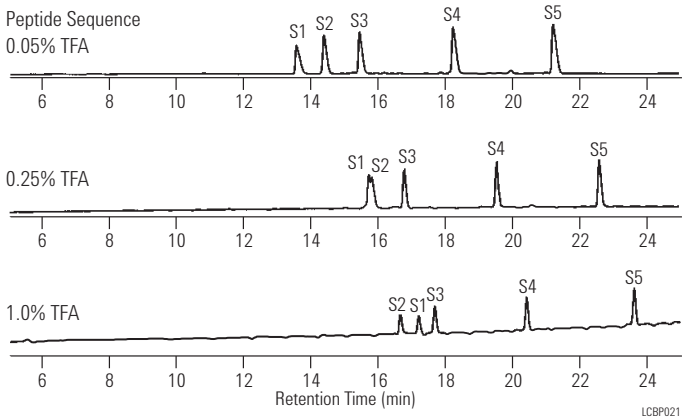
Flow Rate: 1.0 mL/min

Gradient: 0 min 0% B
30 min 30% B

Temperature: 40 °C

Detector: UV, 254 nm

Sample: Peptide Standards S1-S5, decapeptides differing slightly in hydrophobicity, 6 µL



LCBP021

Exploiting chemical stability – TFA concentration

Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

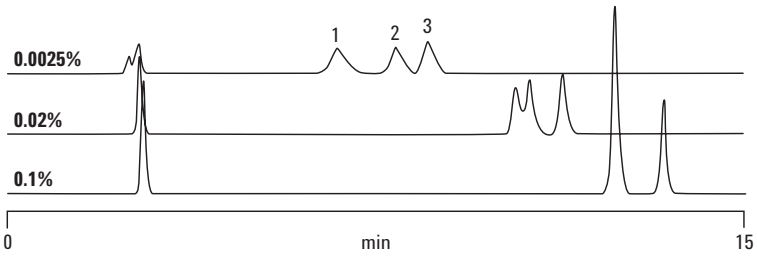
Mobile Phase: A: TFA (various %) in water
B: TFA (various %) in ACN

Gradient: Linear 12-40% B in 15 min

Flow Rate: 1.0 mL/min

Detector: ELS (neb=75 °C, evap=85 °C, gas=1.0 SLM)

1. Angiotensin III
2. Angiotensin II
3. Angiotensin I



VLC0068

Peptides: Separation of Antiotensins I, II, III with TFA and NH_4OH

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

Mobile Phase: A: Acidic Conditions
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
B: Basic Conditions
A: 10 mM NH_4OH in water
B: 10 mM NH_4OH in 80% ACN

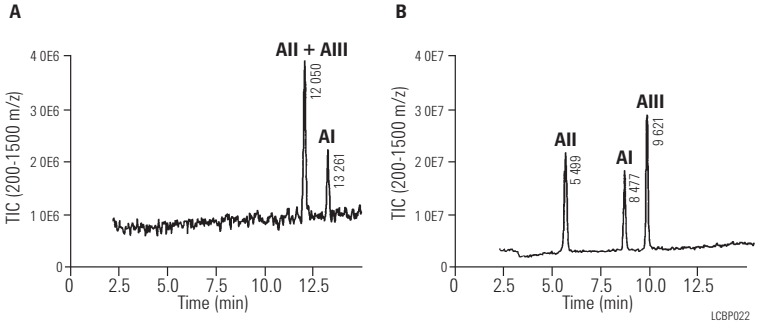
Flow Rate: 0.2 mL/min

Gradient: 15-50% B in 15 min

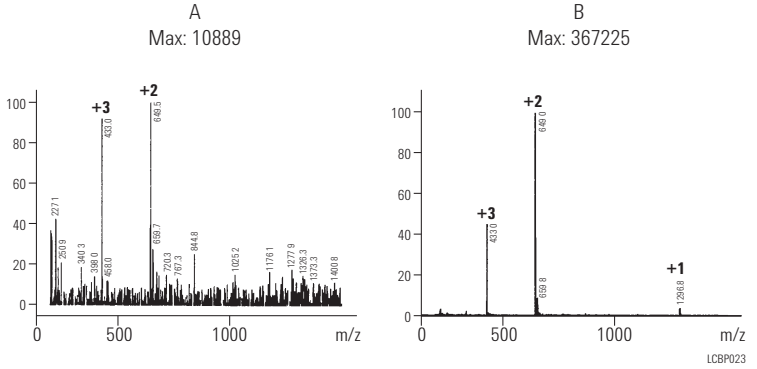
Temperature: 35 °C

MS Conditions: Pos. Ion ESI - Vf 70V, Vcap 4.5 kV
N_2-35 psi, 12 L/min, 325 °C

Sample: 2.5 µL sample (50 pmol each)



LCBP022



LCBP023

Peptides/proteins: Equivalent gradient separations

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

Column: **ZORBAX 300SB-C8**
883750-906
2.1 x 150 mm, 5 µm

Mobile Phase: A: 95% Water: 5% ACN with 0.1% TFA
B: 5% Water: 95% ACN with 0.085% TFA

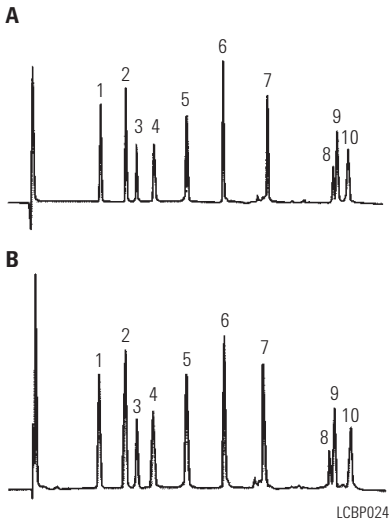
Flow Rate: A: Analytical
1 mL/min
B: Narrow Bore
0.2 mL/min

Gradient: 10-60% B in 30 min

Temperature: 35 °C

Detector: UV, 215 nm

Sample: 10 µL injection, concentration 2-6 µg



LCBP024

1. Met-enkephalin
2. Leu-enkephalin
3. Angiotensin II
4. Neurotensin
5. RNase
6. Insulin (BOV)
7. Lysozyme
8. Calmodulin
9. Myoglobin
10. Carbonic anhydrase

Peptides/proteins: Effect of elevated temperature

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

Mobile Phase: A: 5:95 ACN:Water with 0.10% TFA (v/v%)
B: 95:5 ACN:Water with 0.085% TFA (v/v%)

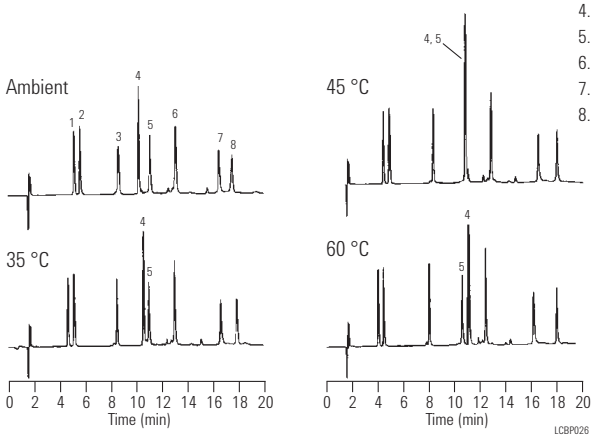
Flow Rate: 1.0 mL/min

Gradient: 15-53% in 20 min, post time 12 min

Temperature: Ambient – 60 °C

Detector: UV, 215 nm

Sample: Polypeptides



LCBP026

1. Leucine Enkephalin
2. Angiotensin II
3. RNase A
4. Insulin (BOV)
5. Cytochrome c
6. Lysozyme
7. Myoglobin
8. Carbonic anhydrase

Separation of polypeptides in under 1 minute

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

Mobile Phase: A: 0.1% TFA, H_2O
B: 0.07% TFA, ACN

Flow Rate: 3 mL/min

Gradient: 0-100% B in 1.33 min

Temperature: 70 °C

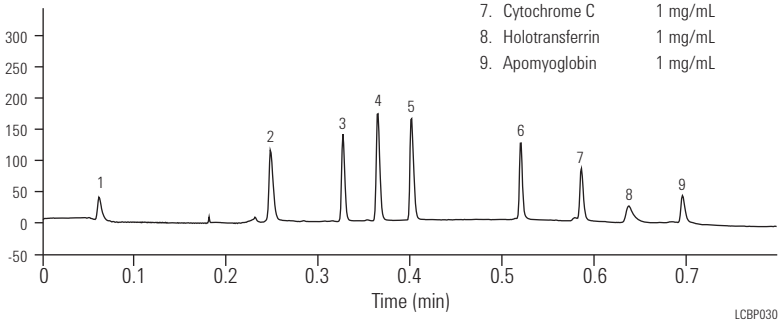
Detector: DAD 215/16 nm, ref = 310/10 nm

Sample: Peptides/proteins, 0.5 µL

Mixer bypassed with P/N G1312-67301; Loop-bypass program

Sample (peptides/proteins)

1.	gly-tyr	0.125 mg/mL
2.	Val-tyr-val	0.5 mg/mL
3.	Met-enkephalin	0.5 mg/mL
4.	Leu-enkephalin	0.5 mg/mL
5.	Angiotensin II	0.5 mg/mL
6.	RNase A	1 mg/mL
7.	Cytochrome C	1 mg/mL
8.	Holotransferrin	1 mg/mL
9.	Apomyoglobin	1 mg/mL



LCBP030

Fast, high-resolution separation of peptides and proteins with Poroshell 300SB-C18

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

Mobile Phase: A: 0.1% TFA
B: 0.07% TFA in ACN

Flow Rate: 3.0 mL/min (360 bar pressure)

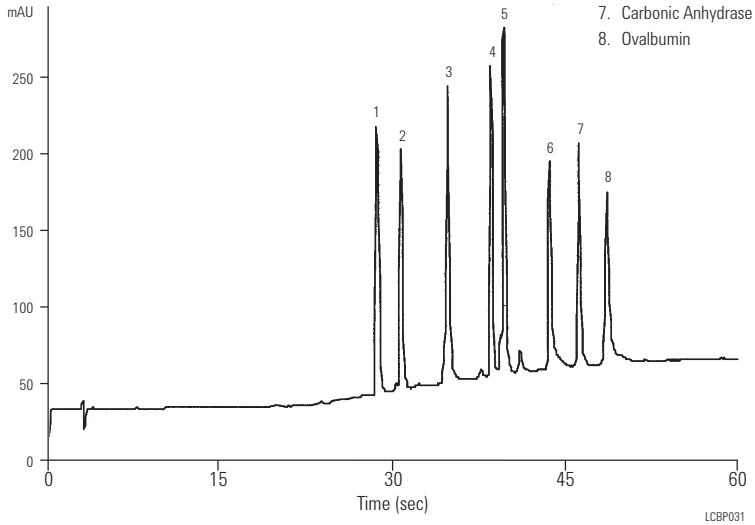
Gradient: 5-100% B in 1.0 min

Temperature: 70 °C

Detector: UV, 215 nm

Spaces between solutes indicate good peak capacity for rapidly separating complex samples.

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



LCBP031

Peptide RP-HPLC/ESI-MS using NH_4OH mobile phase yields both positive and negative ion spectra

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

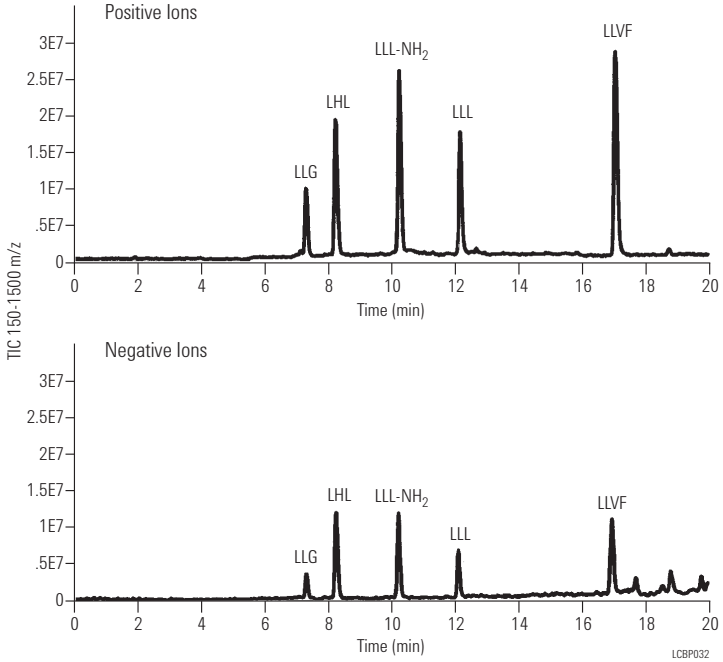
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70 V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min, 300 °C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Comparison of Aβ peptide RP-HPLC separations at low and high pH

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

Mobile Phase: A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN

Flow Rate: 0.25 mL/min

Gradient: 29-41% B in 30 min

Temperature: 80 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)

Mobile Phase: A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

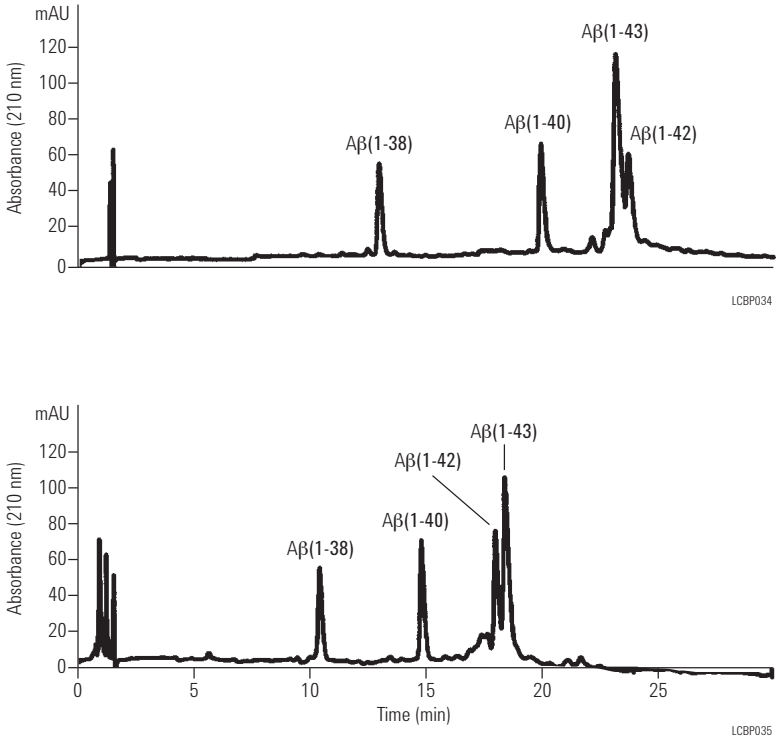
Flow Rate: 0.25 mL/min

Gradient: 26-38% B in 30 min

Temperature: 25 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)



Selectivity comparison of TFA and NH_4OH for peptide RP-HPLC\ESI-MS analysis

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

Mobile Phase: TFA Conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
NH_4OH Conditions:
A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

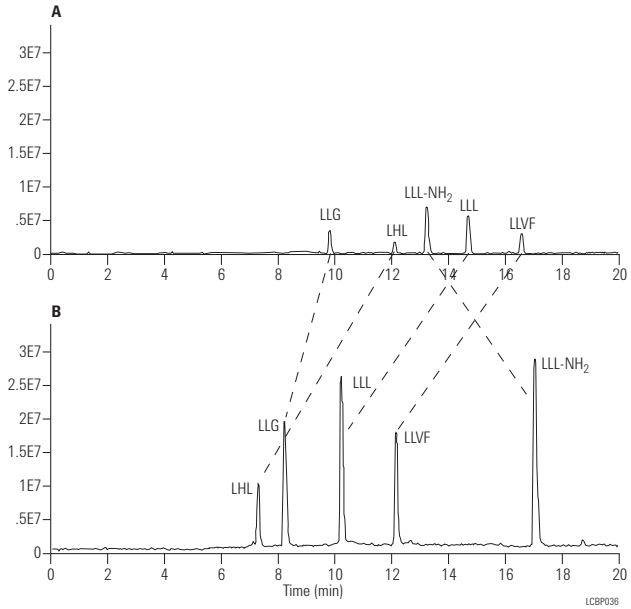
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min., 300 °C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Peptide phosphorylation sites LC and LC/MS using Capillary LC columns

Column: **ZORBAX 300SB-C18**
5064-8268
0.5 x 150 mm, 3.5 µm

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

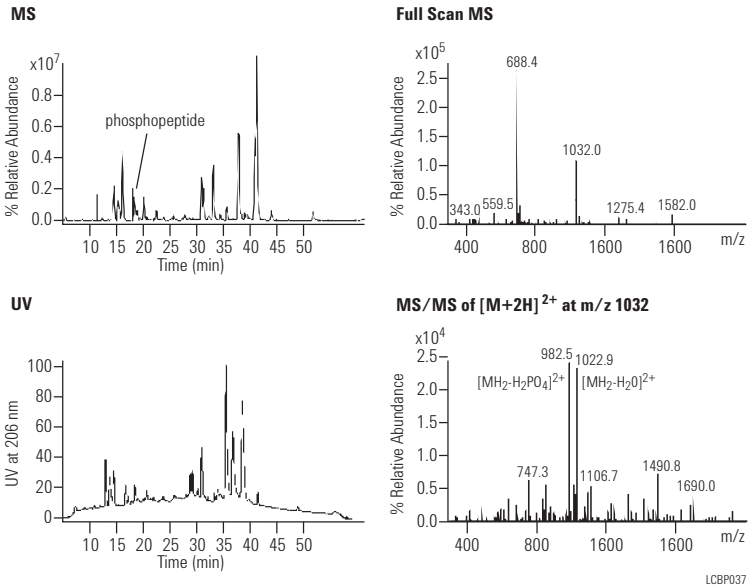
Flow Rate: 5.5 µL/min

Gradient: 5-55% B in 50 min, to
85% B from 55-57 min

Detector: UV, 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 Ampitude: 1.0 V

Sample: Beta case in digest, 100 nL (4 pmol)



Proteins: Effect of bonded phase, RP

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

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

Mobile Phase: A: 0.1% TFA in Water,
B: 0.1% TFA in 50/50 ACN/Water

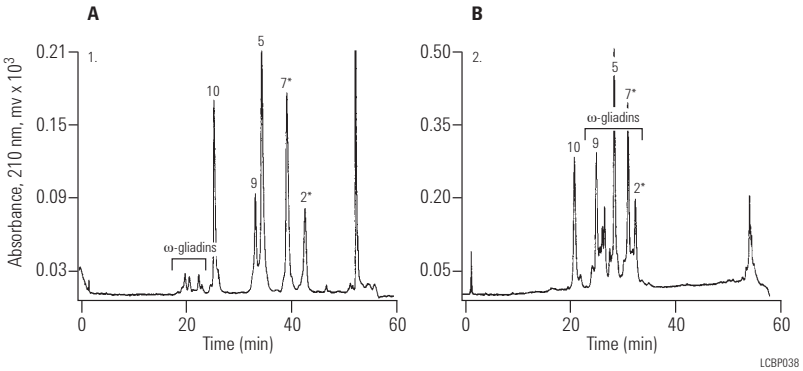
Flow Rate: 1.0 mL/min

Gradient: 1. 46-96% B in 60 min 23-48% ACN
2. 50-86% B in 60 min 25-43% ACN

Temperature: 50 °C

Detector: UV, 210 nm

Sample: Wheat proteins, including w-gliadins



Proteins: Effect of bonded phase

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

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

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

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

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.09% TFA in 80% ACN/20% Water

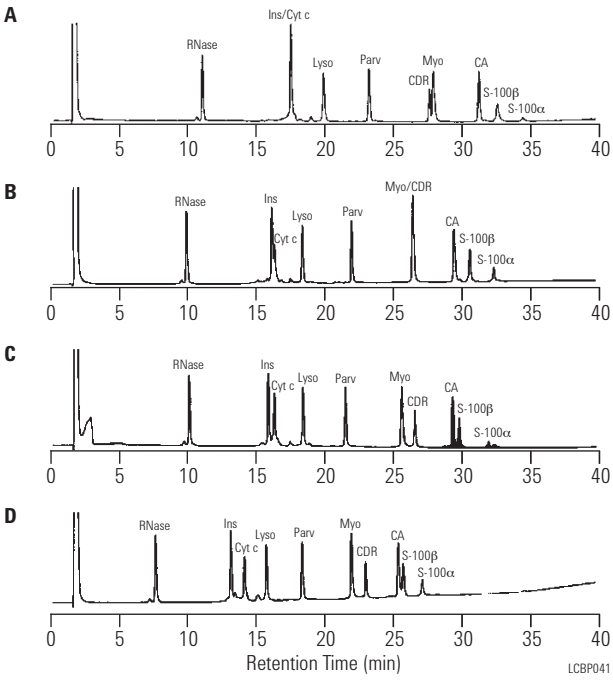
Flow Rate: 1.0 mL/min

Gradient: 25-70% B in 40 min

Temperature: 60 °C

Detector: UV, 210 nm

Sample: Polypeptides, 3 µg each



Standard proteins by reversed-phase

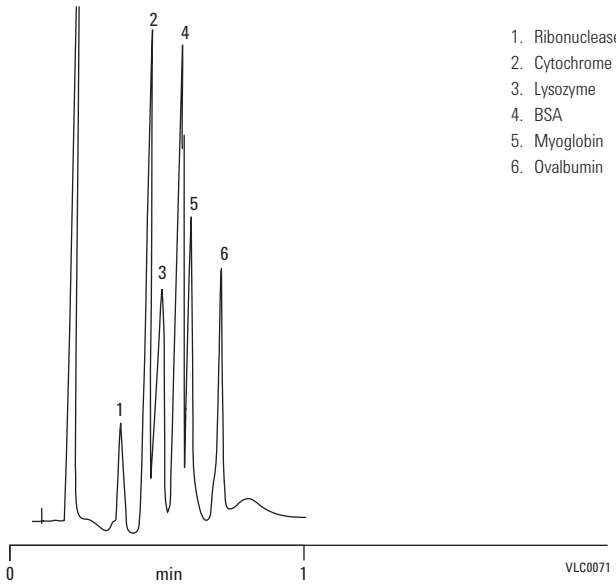
Column: **PLRP-S 4000Å**
PL1512-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.1% TFA in 95% water:5% ACN
B: 0.1% TFA in 5% water:95% ACN

Gradient: Linear 18-60% B in 1 min

Flow Rate: 4.0 mL/min

Detector: UV, 280 nm



1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. Myoglobin
6. Ovalbumin

Standard ion-exchange protein separation

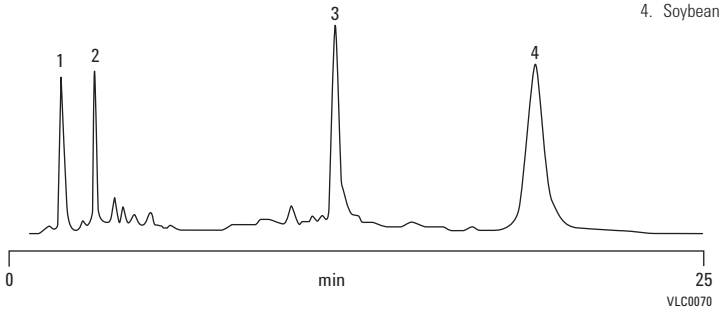
Column: **PL-SAX 1000Å**
PL1551-1502
4.6 x 50 mm, 5 µm

Mobile Phase: A: 10 mM Tris HCl pH 8
B: A+0.35 M NaCl pH 8

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



1. Myoglobin
2. Bovine carbonic anhydrase
3. Ovalbumin
4. Soybean trypsin inhibitor

Deoxynucleosides: Using rapid resolution 3.5 µm columns

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

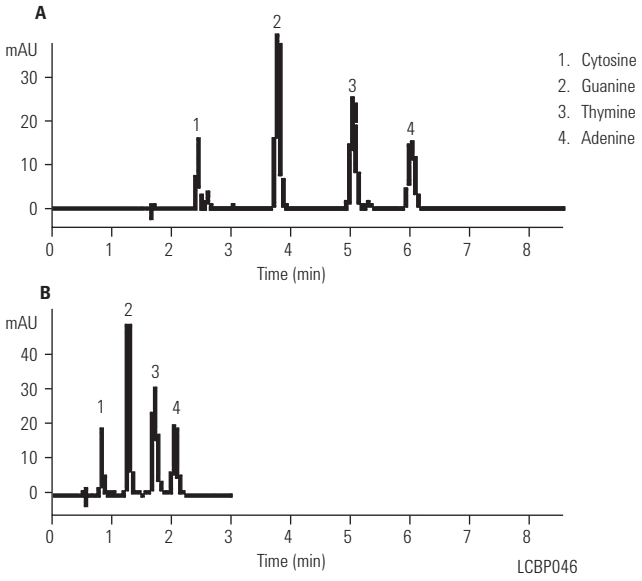
Column B: **ZORBAX SB-CN**
835975-905
4.6 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA
B: 90/10 v/v Methanol/Water (0.1% TFA)
Isocratic, 97.5% A, 2.5% B

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 254 nm



BSA tryptic digest on RRHT

Column: **ZORBAX SB-C18**
820700-902
2.1 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

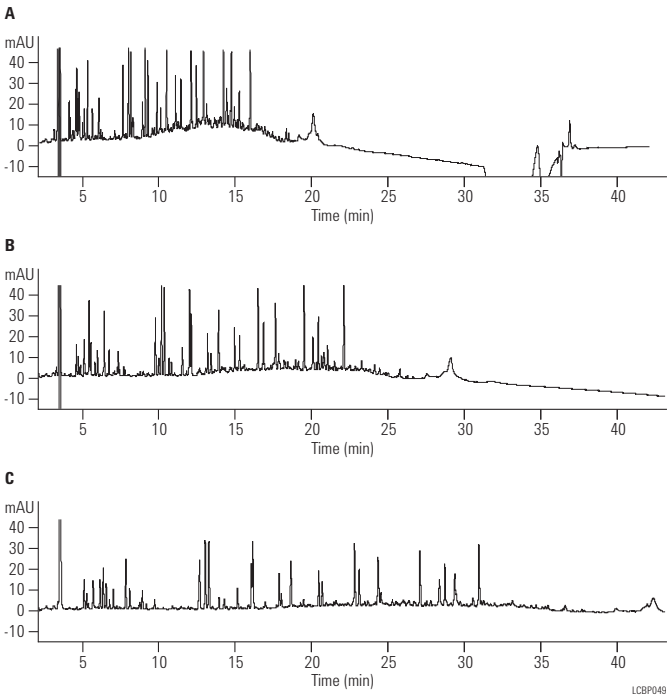
Flow Rate: 0.5 mL/min

Gradient: A: Time 0% B 5 min, Time 30% B 60 min
B: Time 0% B 5 min, Time 45% B 60 min
C: Time 0% B 5 min, Time 67.5% B 60 min

Temperature: 80 °C

Detector: UV, 214 nm

Sample: BSA tryptic digest



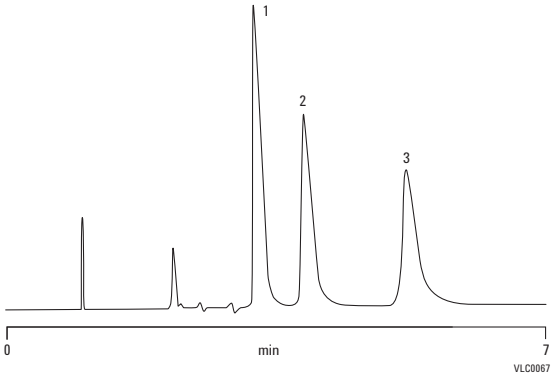
Catecholamines

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: 95% 25 mM citric acid, 25 mM Na_2HPO_4, 1 mM heptane sulfonic acid:5% ACN, pH 2.85

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Noradrenaline
2. Adrenaline
3. Dopamine

Whey proteins in dairy samples – milk

Column: **PLRP-S 300Å**
PL1512-3801
4.6 x 150 mm, 8 µm

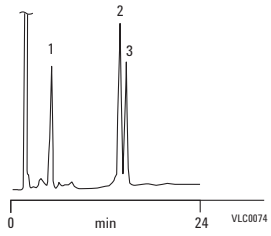
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

Gradient: 36-48% B, 0-24 min, 48-100% B, 24-30 min
100% B, 30-35 min, 100-36% B, 35-40 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Detector: UV, 220 nm



1. α-Lactalbumin
2. β-Lactoglobulin (B chain)
3. β-Lactoglobulin (A chain)

Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion-pair reversed-phase HPLC

Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

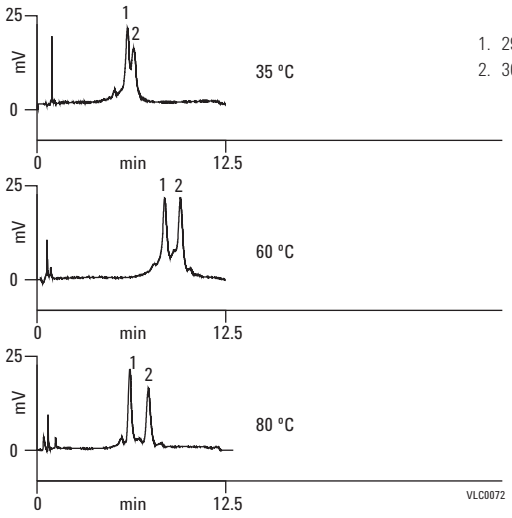
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

Gradient: 5% change in buffer B over 5 min

Flow Rate: 1.0 mL/min

Temperature: 35 °C, 60 °C, or 80 °C

Detector: UV, 254 nm



1. 29-mer
2. 30-mer

Hydrophilic purine/pyrimidine separation

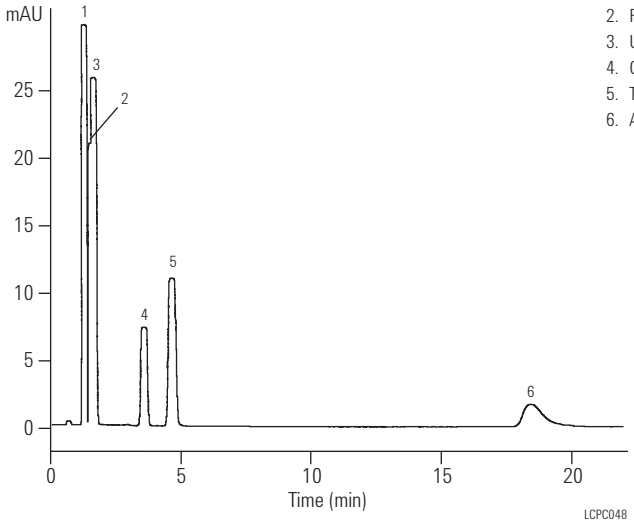
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 50 mM NaOAc, pH 4.6

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



1. Cytosine
2. Fluorocytosine
3. Uracil
4. Guanine
5. Thymine
6. Adenine

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Chemical/Industrial Applications

Analysis of biocides in hand sanitizer

Column: **ZORBAX RRHD Eclipse Plus C18**
959757-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: H_2O (0.5% TFA)
B: ACN (0.04% TFA)

Flow Rate: 1.7 mL/min

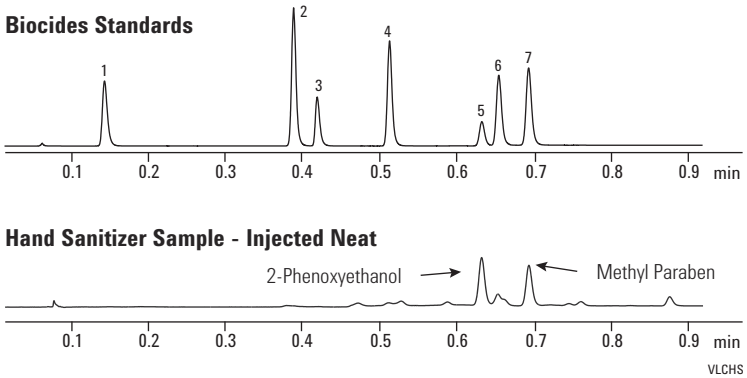
Gradient:

Time 0.0	95/5 A/B	
Time 1.0	55/45 A/B	
Time 1.1	0/100 A/B	

DAD: 275 nm (0 min)
225 nm (0.46 min)
255 nm (0.67 min)

Sample: 1 µL injection of 50 ppm std.

Temperature: 30 °C



1. Kathon 1A
2. Kathon 1B
3. Carbendazim
4. 1,2-Benzisothiazol-3(2H)-one
5. 2-Phenoxyethanol
6. Benzoic Acid
7. Methyl Paraben

Triton X-114: Decreasing run-time by changing bonded phase

Column A: **ZORBAX SB-C3**
883975-909
4.6 x 150 mm, 5 µm

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

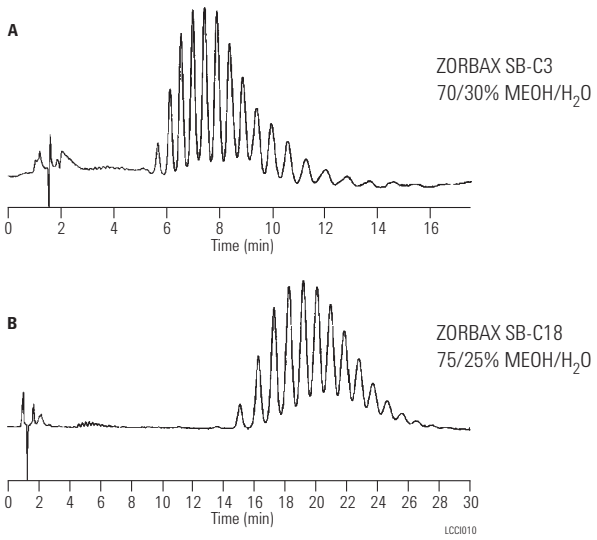
Mobile Phase: MeOH and H_2O (as indicated)

Flow Rate: 1.0 mL/min

Temperature: 50 °C

Detector: UV, 225 nm

Sample: Triton X-114



Organic acids separated on ZORBAX SB-Aq

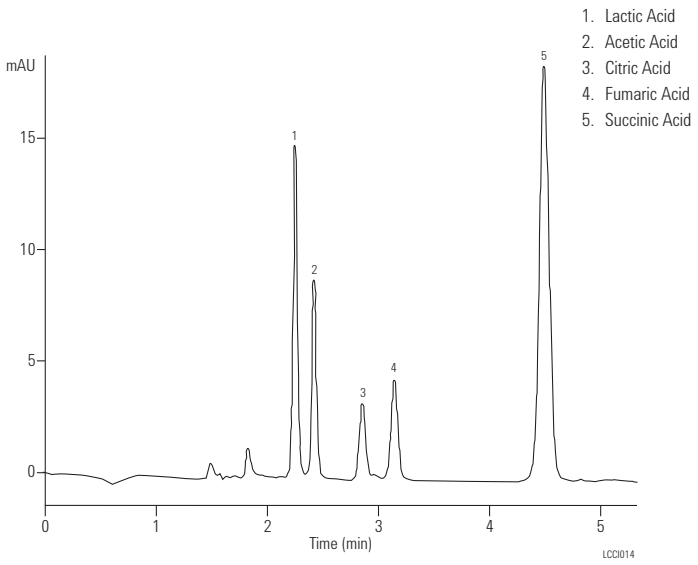
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 99% 20 mM NaH_2PO_4, pH 2, 1% ACN

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 210 nm



Brij 35

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: A: Water
B: ACN

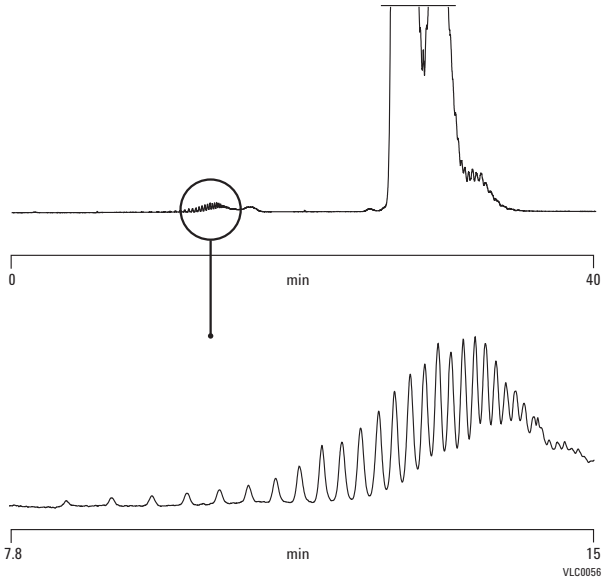
Gradient: 0-100% B in 40 min

Flow Rate: 0.8 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

Detector: ELS (neb=50 °C, evap=70 °C, gas=1.5 SLM)



Alcohols and aliphatic compounds

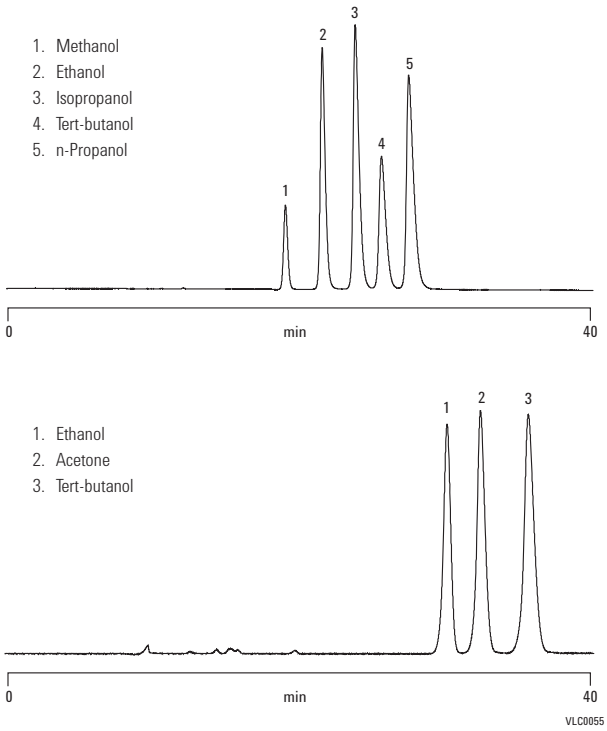
Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 40 °C

Detector: 356-LC RI



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Environmental Applications

NEW!

Fast LC/MS/MS analysis of group 4 pharmaceuticals from EPA-1694

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

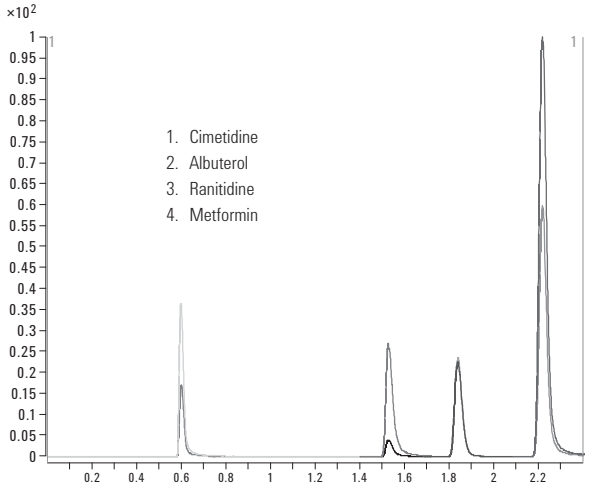
Mobile Phase: A: 10 mM ammonium acetate in water, pH 6.7
B: acetonitrile

Flow Rate: 1 mL/min

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

MS Conditions: TCC: 25 °C
dMRM, ESI positive mode, cycle time 35 ms
Drying Gas: 9 L/min, 300 °C
Nebulizer Pressure: 40 psig
Capillary Voltage: 4000

Sample: 0.1 µL injection of 0.1 mg/mL each in acetonitrile/water (3:1): cimetidine, albuterol, ranitidine and metformin



NEW!

Separation of azo dye degradation products

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

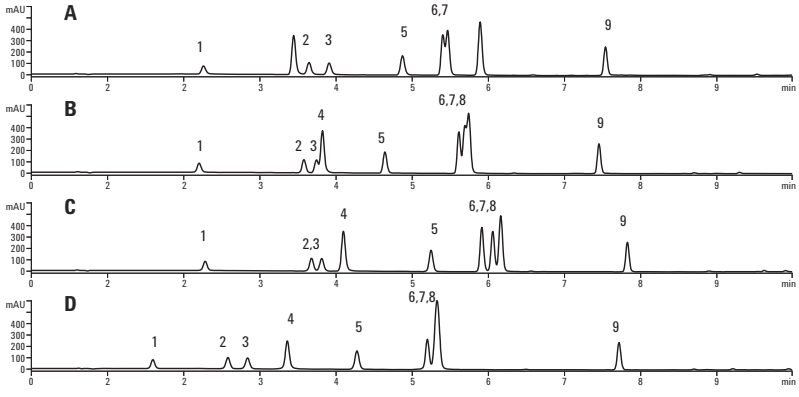
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Flow Rate: 0.4 mL/min

Gradient: 15 to 100% MeOH over 10 min

Solvent: 10 mM Ammonium acetate, pH 4.8

1. Aniline
2. o-Toluidine
3. Methoxyaniline
4. Chloroaniline
5. Benzidine
6. Dimethylbenzidine
7. 3,3'-Dimethoxybenzidine
8. Napthylamine
9. Dichlorobenzidine



Comparison of phenols separation with Poroshell 120

Column: **Poroshell 120 EC-C18**
699975-902
4.6 x 50 mm, 2.7 µm

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
0.8	5%
6.8	60%

1200 SL controlled temperature
at 25 °C 2 mm flow cell

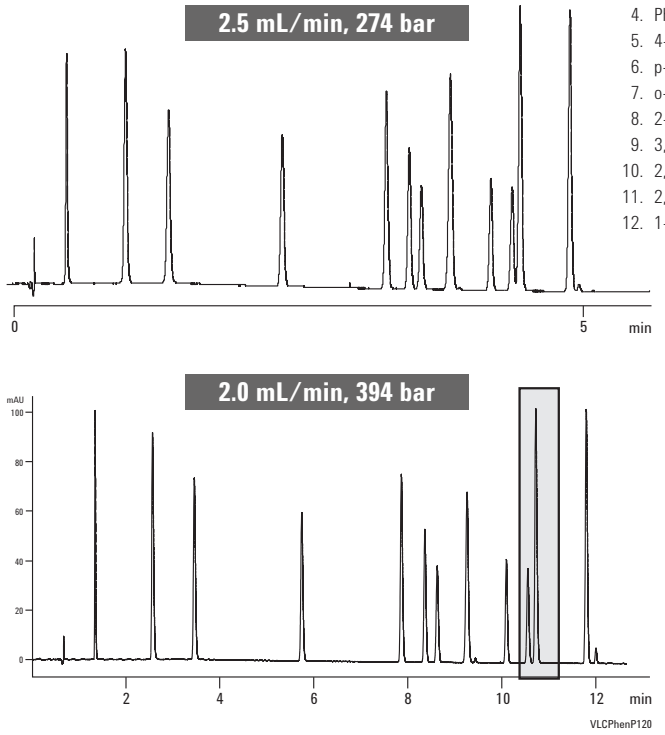
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
2.0	5%
17	60%

1200 RRLC SL controlled temperature
at 25 °C 2 mm flow cell



1. Hydroquinone
2. Resourcinol
3. Catechol
4. Phenol
5. 4-Nitrophenol
6. p-cresol
7. o-cresol
8. 2-Nitrophenol
9. 3,4 di methyl phenol
10. 2,3 di methyl phenol
11. 2,5 di methyl phenol
12. 1-napthol

DNPH: Derivatized Aldehydes obtained from air

Column: **ZORBAX ODS**
884950-543
4.6 x 250 mm, 5 µm

Mobile Phase: A: 100% Water
B: 100% ACN

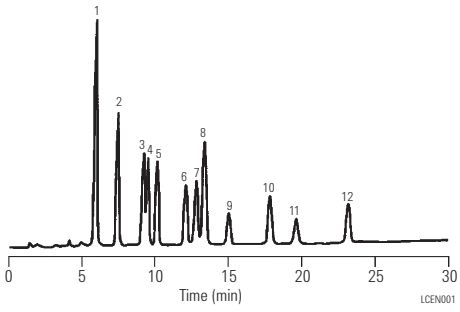
Flow Rate: 1.0 mL/min

Gradient: 60-75% B in 30 min; Wash: From 75-100% B in 5 min, after 5 min return to 60% B

Temperature: 35 °C

Detector: UV, 230 nm

Sample: DNPH Derivatized Aldehydes



1. Formaldehyde – DNPH
2. Acetaldehyde – DNPH
3. Acetone – DNPH
4. Acrolein – DNPH
5. Propionaldehyde – DNPH
6. Crotonaldehyde – DNPH
7. 2-Butanone (MEK) – DNPH
8. Methacrolein – DNPH
   n-Butyraldehyde – DNPH
9. Benzaldehyde – DNPH
10. Valeraldehyde – DNPH
11. m-Tolualdehyde – DNPH
12. Hexaldehyde – DNPH

Amitrol in water by LC/MS, 0.05 ppb

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

Mobile Phase: A: 10 mM ammonium acetate
B: MeOH

Flow Rate: 0.4 mL/min

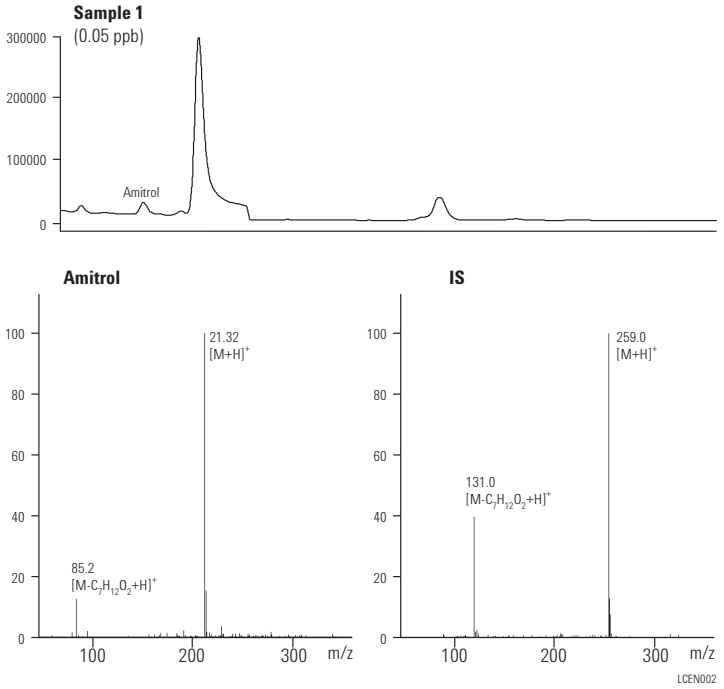
Gradient: 0 min, 65% B; 10 min, 65% B;
15 min, 100% B; 20 min, 65% B

Temperature: 30 °C

MS Conditions:

Ionization Mode:	APCI, positive polarity
SIM parameters:	Ion: 213 Amitrol
Ion:	259 IS
Fragmentor:	100 V
SIM Resolution:	Low
Vaporizer:	325 °C
Drying Gas (N_2):	5.0 L/min
Gas Temperature:	350 °C
Nebulizer pressure:	60 psig
Vcap:	4000 V
Corona:	4.0 uA

Sample: Amitrol in water, 100 µL



Anilines, substituted: Rapid separation

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

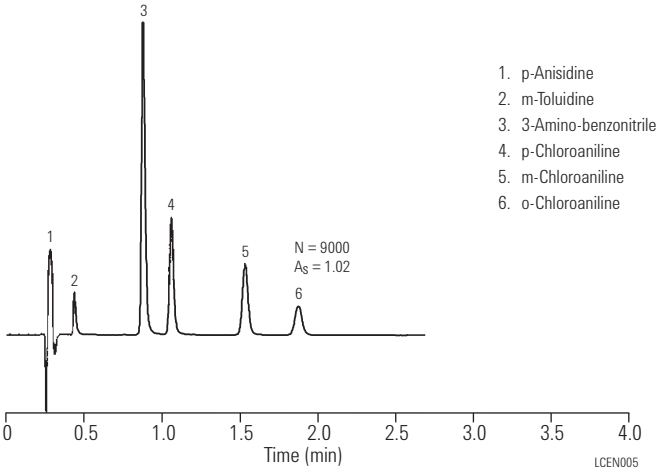
Mobile Phase: 20% ACN/80% 25 mM phosphate buffer, pH 2.5

Flow Rate: 3.0 mL/min

Temperature: 60 °C

Detector: UV, 254 nm

Sample: Anilines



1. p-Anisidine
2. m-Toluidine
3. 3-Amino-benzonitrile
4. p-Chloroaniline
5. m-Chloroaniline
6. o-Chloroaniline

Explosives and related compounds: Qualitative and quantitative analysis

Column A: **ZORBAX SB-C18**
883700-922
2.1 x 150 mm, 5 µm

Column B: **ZORBAX SB-CN**
883700-905
2.1 x 150 mm, 5 µm

Mobile Phase: A = ACN + 5% H_2O + 5 mM CF_3COONH_4
B = H_2O + 5% ACN + 5 mM CF_3COONH_4,
pH 2.7 (CF_3COOH)

Flow Rate: 0.23 mL/min

Gradient: A:
0 min 80% B
2 min 80% B
10 min 70% B
20 min 65% B
25 min 60% B
35 min 30% B
40 min 30% B
42 min 80% B

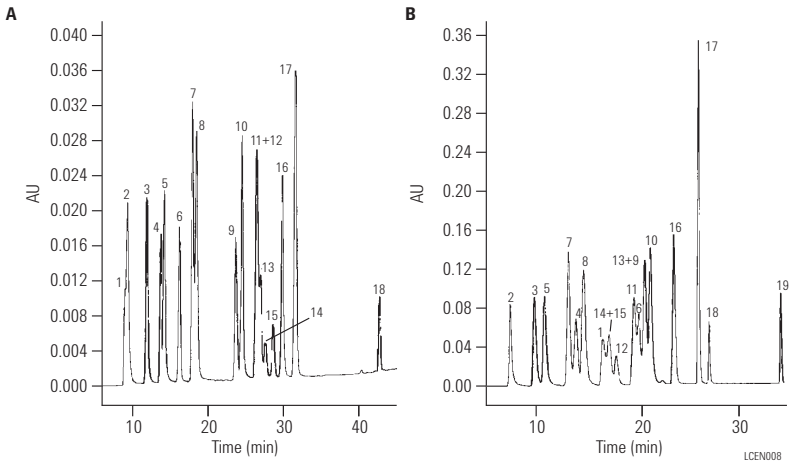
B:
0 min 80% B
1 min 80% B
15 min 70% B
30 min 20% B
35 min 20% B
37 min 80% B

Temperature: 18 °C

Detector: UV, 210, 240, 360 nm, wavelength switching for each compound

Sample: 10 µL of 19 explosive compounds in ACN/H_2O (20/80)

1. Picric acid
2. 4-Amino-2-nitrotoluene
3. 2-Amino-6-nitrotoluene
4. RDX
5. 2-Amino-4-nitrotoluene
6. HMX
7. 1,3-Dinitrobenzene
8. 1,3,5-Trinitrobenzene
9. 2-Amino-4,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 4-Amino-4,6-dinitrotoluene
12. 2-Nitrotoluene
13. 2,6-Dinitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene
16. 2,4,6-Trinitrotoluene
17. Tetryl
18. Diphenylamine
19. Hexyl



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Explosives from soil extract

Column: **ZORBAX SB-C18**
880975-302
3.0 x 250 mm, 5 µm

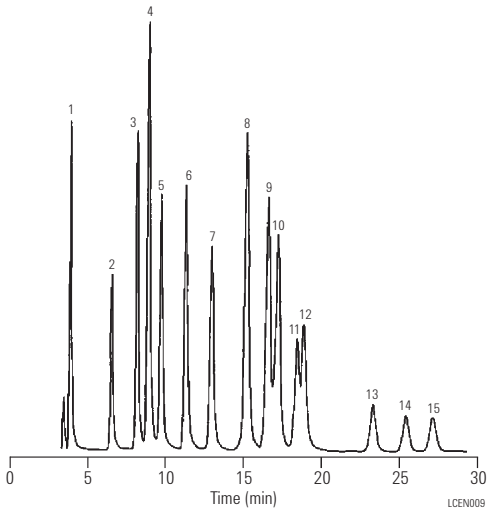
Mobile Phase: Methanol/Water (50/50) (v/v)

Flow Rate: 0.3 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 10 µL explosives mix



1. Octogen (HMX)
2. Hexogen (RDX)
3. 2-Amino-6-nitrotoluene
4. 1,3,5-Trinitrobenzene
5. 2-Amino-4-nitrotoluene
6. 1,3-Dinitrobenzene
7. Tetryl
8. 2,4,6-Trinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2-Nitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene

Herbicides on different bonded phases

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

Column B: **ZORBAX SB-Phenyl**
883975-912
4.6 x 150 mm, 5 µm

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

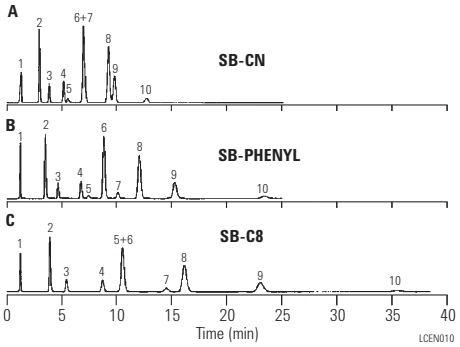
Mobile Phase: 35% ACN, 65% Water

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

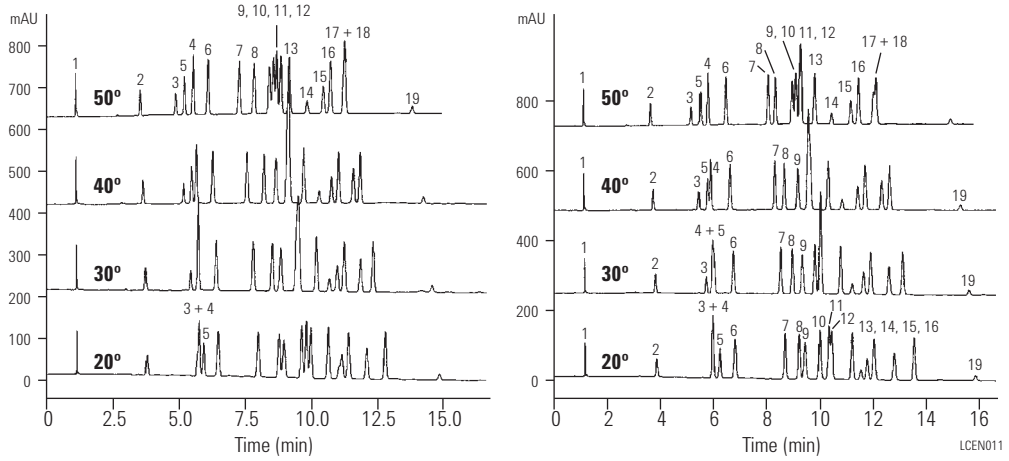
Sample: Herbicides



1. Bentazon
2. Tebuthiuron
3. Simazine
4. Atrazine
5. Prometon
6. Diuron
7. Propazine
8. Propanil
9. Prometryne
10. Metolachlor

Herbicide/pesticide standards: Effect of bonded phase

	Column: Eclipse XDB-C8 993967-906 4.6 x 150 mm, 5 µm	Column: Eclipse XDB-C18 993967-902 4.6 x 150 mm, 5 µm
Mobile Phase:	Water/Acetonitrile	Water/Acetonitrile
Flow Rate:	1.0 mL/min	1.0 mL/min
Gradient:	20-60% in 15 min	20-60% in 15 min
Temperature:	50 °C 40 °C 30 °C 20 °C	50 °C 40 °C 30 °C 20 °C
Detector:	DAD 240	DAD 240
Sample:	Herbicide & pesticide standards	Herbicide & pesticide standards



1. Desethyldesisopropylatrazine
2. Desethylatrazine
3. Benzthiazuron
4. Hexazinon
5. Metoxuron
6. Simazine
7. Methabenzthiazuron
8. Simazine
9. Atrazine
10. Isoproturon
11. Diuron
12. Monolonuron
13. Metobromuron
14. Metazachlor
15. Propazine
16. Sebutylazine
17. Terbutylazine
18. Linuron
19. Metolachlor

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Separation of EPA 610 PAH Mix

Column: **Eclipse PAH**
959990-318
3.0 x 250 mm, 5 µm

Mobile Phase: A: Water
B: Acetonitrile
Initial %B = 40

Flow Rate: 0.85 mL/min

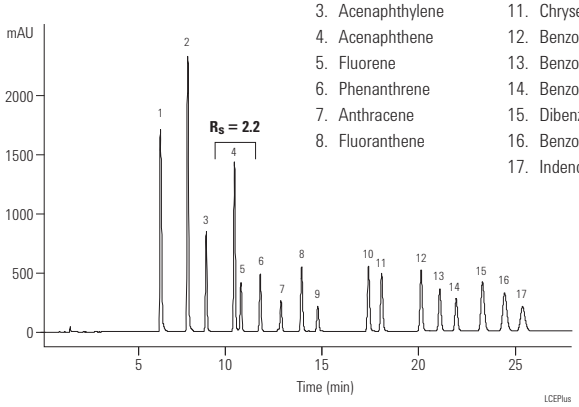
Gradient:

Time (Min)	%B
0.00	45
17.5	100
24.0	100
25.5	40
27.5	40

Stop Time = 25.0

Temperature: 25 °C

Detector: 220, 4 nm No Ref.; Stop time = 26.0 min



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benzo(a)anthracene
11. Chrysene
12. Benzo(b)fluoranthene
13. Benzo(k)fluoeanthene
14. Benzo(a)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(g,h,i)perylene
17. Indeno(1,2,3-c,d)pyrene

Polycyclic aromatic hydrocarbons according to EPA Method 610

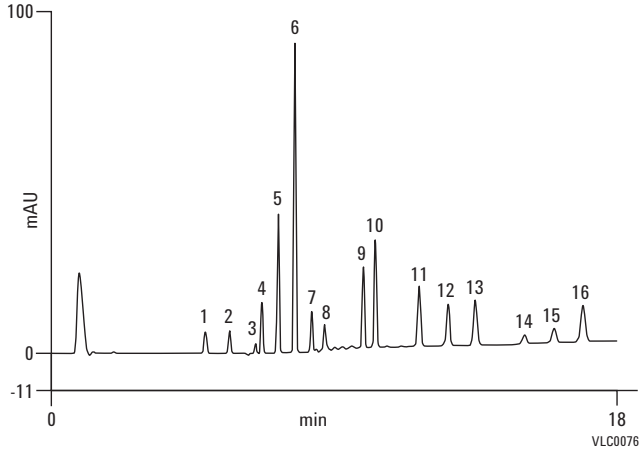
Column: **Pursuit PAH**
A7001100X046
4.6 x 100 mm, 3 µm

Sample: NIST 16473 Standard

Mobile Phase: A: ACN:water, 25:75
B: ACN

Flow Rate: 2.0 mL/min

Detector: UV, 254 nm



1. Naphthalene
2. Acenaphthylene
3. Aceneaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenzo[a,h]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene

NEW!

Rapid method development for 18 PAH compounds with an Agilent RRHD Eclipse PAH column

Column: **ZORBAX RRHD Eclipse PAH**
959758-918
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: Water
B: Acetonitrile

Flow Rate: 0.84 mL/min

Gradient: 40-100% B, gradient time (t_g) varies from 1 to 20 min; isocratic hold at 100% B for 2 min, re-equilibrate column at 40% B for 3 min

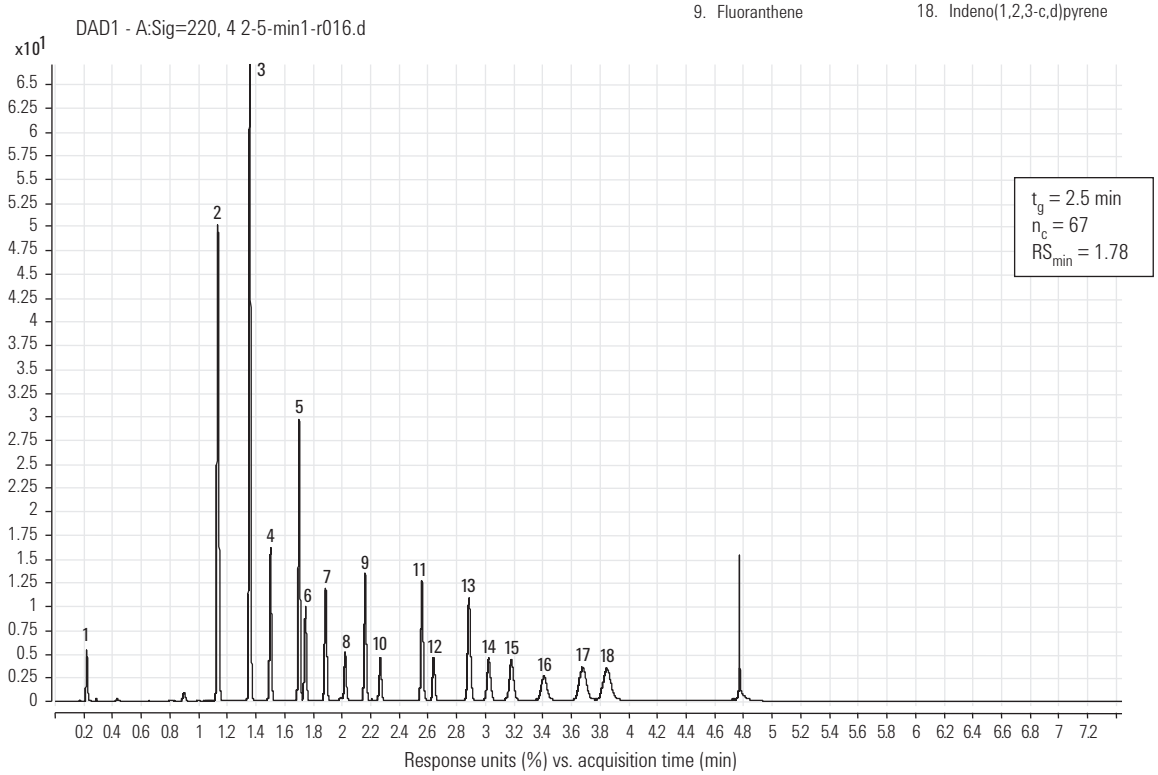
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC

MS Conditions: Sig = 220, 4 nm; Ref = Off

Sample: 0.5 µL injection of diluted Agilent PAH Mixture (P/N 8500-6035) spiked with thiourea as a v_0 marker

1. Thiourea (V_0 marker)
2. Toluene
3. Naphthalene
4. Acenaphthylene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo(b)fluoranthene
14. Benzo(k)fluoranthene
15. Benzo(a)pyrene
16. Dibenzo(a,h)anthracene
17. Benzo(g,h,i)perylene
18. Indeno(1,2,3-c,d)pyrene



Gradient times are rapidly screened for the separation of 18 compounds.

Separation of 20 PAHs on Eclipse PAH

Column: **Eclipse PAH**
959964-918
4.6 x 100 mm, 1.8 µm

Mobile Phase: A: Water
B: Acetonitrile

Flow Rate: 1.8 mL/min

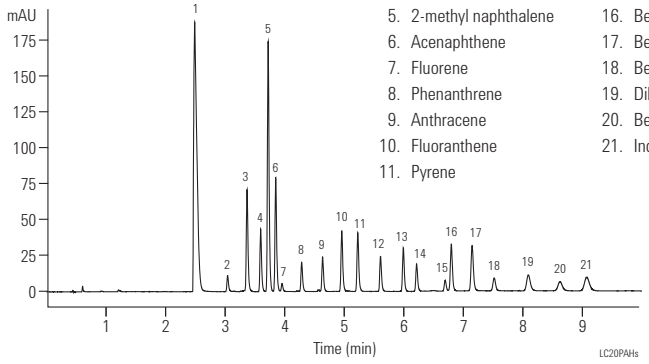
Gradient:

Time (Min)	% B
0	40
6	100
9.5	100
10	40

Stop Time = 12

Temperature: 25 °C

Detector: 230, 8 nm No Ref.; Data rate 0.2 s, micro flow cell



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. 1-methyl naphthalene
5. 2-methyl naphthalene
6. Acenaphthene
7. Fluorene
8. Phenanthrene
9. Anthracene
10. Fluoranthene
11. Pyrene
12. Terphenyl-d14
13. Benzo(a)anthracene
14. Chrysene
15. Benz(e)pyrene
16. Benzo(b)fluoranthene
17. Benzo(k)fluoranthene
18. Benzo(a)pyrene
19. Dibenzo(a,h)anthracene
20. Benzo(g,h,i)perylene
21. Indeno(1,2,3-c,d)pyrene

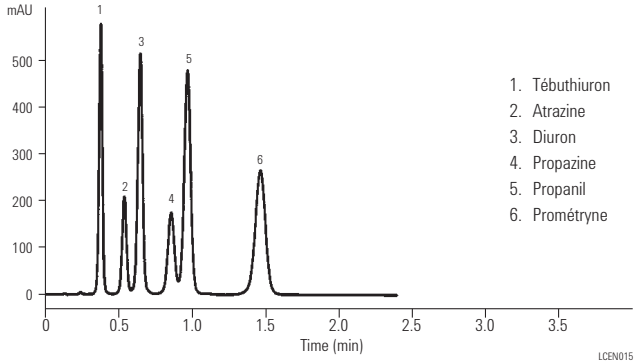
Herbicides: Rapid separation

Column: **Eclipse XDB-C18**
933975-902
4.6 x 30 mm, 3.5 µm

Mobile Phase: MeOH:H_2O (60:40)

Flow Rate: 2 mL/min

Temperature: Ambient



1. Tébuthiuron
2. Atrazine
3. Diuron
4. Propazine
5. Propanil
6. Prométryne

Phenoxyacid herbicides

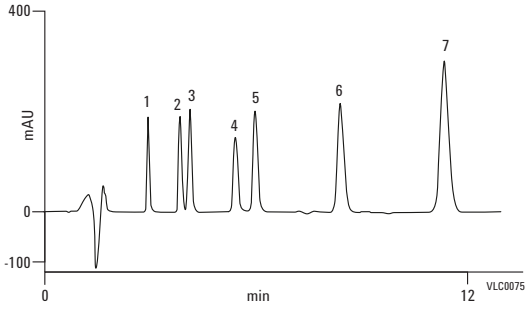
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: MeCN:water+0.1% HCOOH, 50:50

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm



1. Phenoxyacetic acid
2. o-Chlorophenoxyacetic acid
3. p-Chlorophenoxyacetic acid
4. 2,3-Dichlorophenoxyacetic acid
5. 2,4-Dichlorophenoxyacetic acid.
6. 2,4,5-Trichlorophenoxyacetic acid
7. 2,4,5-Trichlorophenoxypropionic acid (Silvex)

Triazine pesticides on Bonus-RP and Alkyl C8 phase

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

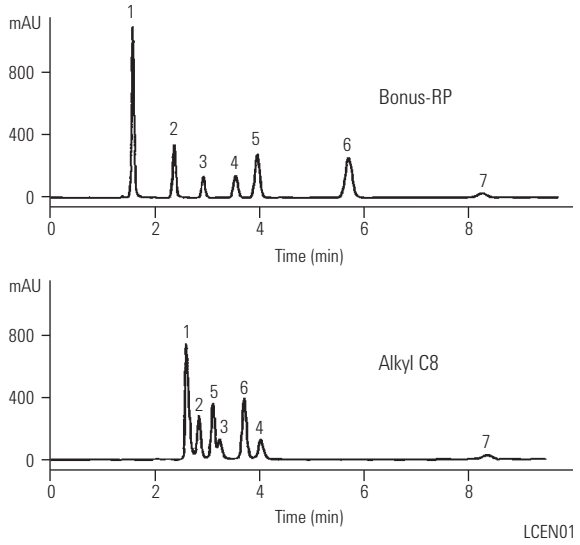
Mobile Phase: MeOH: 0.1% TFA (70:30)*

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 254 nm

Sample: Triazine pesticides, 2 µL
1. Prometryne
2. Tebuthiuron
3. Atrazine
4. Propazine
5. Diuron
6. Propanil
7. Dacthal



LCEN01

* For low pH work with Bonus-RP, a TFA mobile phase is often preferred over phosphate, and is compatible with LC/MS.

Phenols, substituted

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

Mobile Phase: 20% ACN/80% 0.01 M H_3PO_4 to 45% ACN in 7.5 min

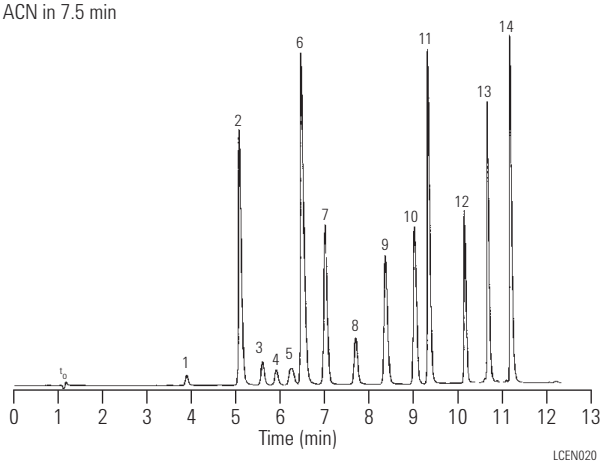
Flow Rate: 1.5 mL/min

Gradient: 80% ACN in 2.0 min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Phenols



LCEN020

1. Phenol
2. 4-Nitrophenol
3. m-Cresol
4. o-Cresol
5. 2-Chlorophenol
6. 2,4-Dinitrophenol
7. 2-Nitrophenol
8. 2,4-Dimethylphenol
9. 4-Chloro-3-methylphenol
10. 2,4-Dichlorophenol
11. 2-Methyl-4,6-dinitrophenol
12. 2,4,6-Trichlorophenol
13. 2,3,4,6-Tetrachlorophenol
14. Pentachlorophenol

Plant hormones: Rapid gradient elution separation

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

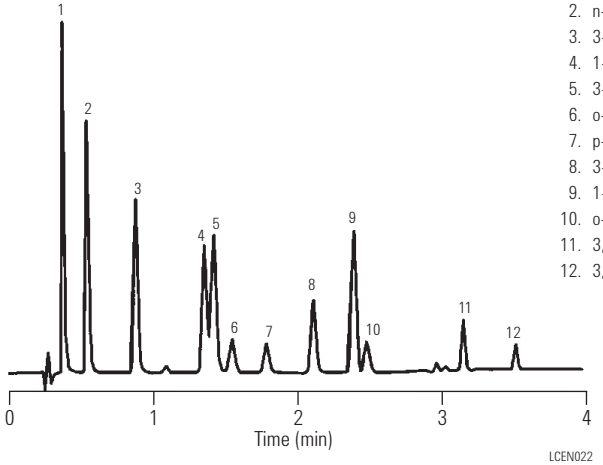
Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

Flow Rate: 3.0 mL/min

Temperature: 60 °C

Detector: UV, 245 nm

Sample: Plant hormones



1. Kinetin
2. n-6-Benzyl adenine
3. 3-Indole acetic acid
4. 1-Naphthyl acetamide
5. 3-Indole proprionic acid
6. o-Chlorophenoxy acetic acid
7. p-Chlorophenoxy acetic acid
8. 3-Indole butyric acid
9. 1-Naphthyl acetic acid
10. o-Chlorophenoxy proprionic acid
11. 3,4,5-Trichlorophenoxy acetic acid
12. 3,4,5-Trichlorophenoxy proprionic acid

VX nerve agent metabolites by LC/MS-IS standard (C13 labeled)

Column: **ZORBAX NH2**
860700-708
2.1 x 50 mm, 5 µm

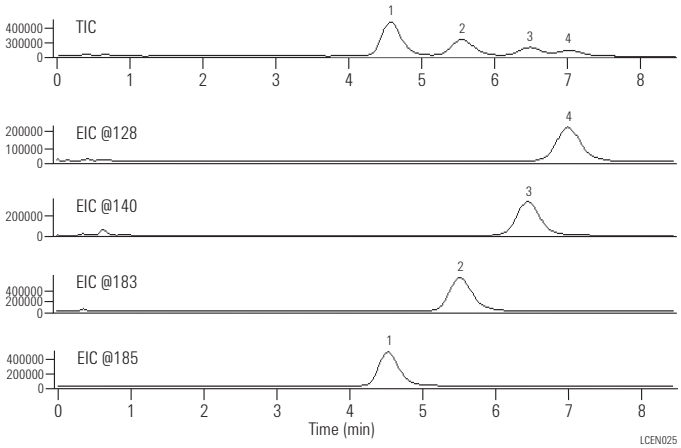
Mobile Phase: 1:1 (20 mM Ammonium Actetate pH 4.5/Acetonitrile)

Flow Rate: 0.5 mL/min, 1 µL injection (prepared std in ACN)

Temperature: 35 °C

Detector: ESI-Negative Ion, Gas Flow 12 L/min, Nebulizer 60 psi

Sample	MW
1. Cyclohexyl methylphosphonic acid	178
2. Pinacolyl methylphosphonic acid	180
3. Isopropyl methylphosphonic acid	138
4. Ethyl methylphosphonic acid	124



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Food and Consumer Product Applications

NEW!

Blueberry anthocyanin analysis

Column A: **Poroshell 120 SB-C18**
687975-902
4.6 x 75 mm, 2.7 µm

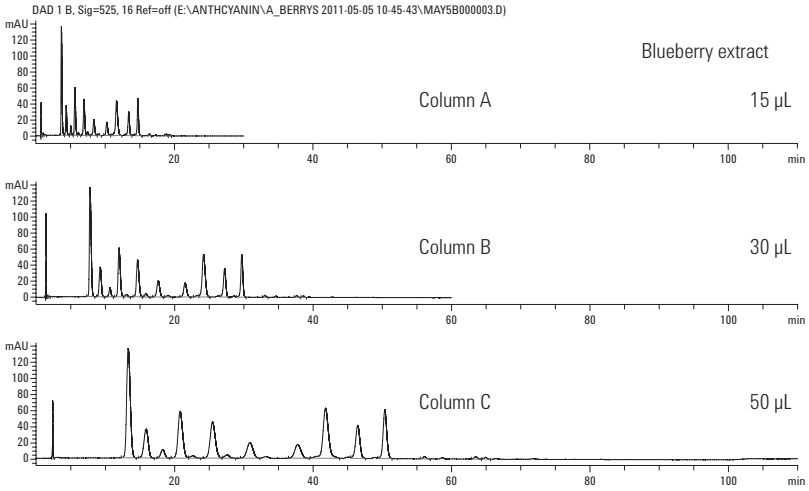
Column B: **ZORBAX SB-C18**
863953-902
4.6 x 150 mm, 3.5 µm

Column C: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

Flow Rate: 1 mL/min

Detector: Agilent 1260 Rapid Infinity LC

Blueberry anthocyanin analysis on totally porous and superficially porous StableBond C18 columns. Overlay of anthocyanin method with 250 mm 5 µm, 150 mm 3.5 µm, and 75 mm 2.7 µm at 1 mL/min.



NEW!

Analysis of pesticide residues in green tea

Column: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Mobile Phase: A: 5 mM FA in water
B: 5 mM FA in ACN

Flow Rate: 0.4 mL/min

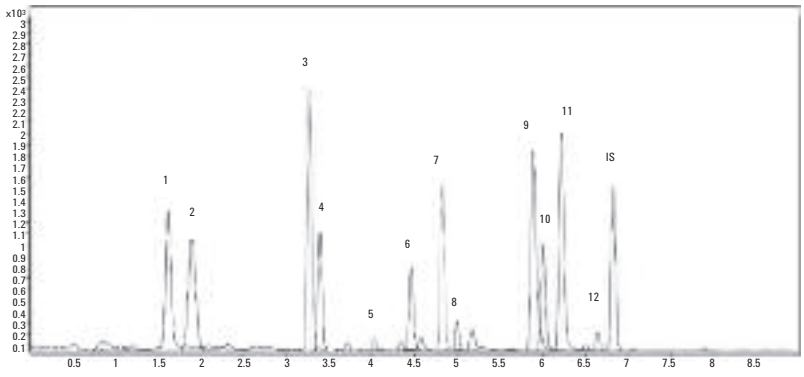
Gradient: 5% B in 1 min, 50% B in 3 min, 90% B in 7 min, 90% B in 8 min, 5% B in 8.2 min, 5% B in 9 min

Temperature: 30 °C

MRM chromatograms of 50 ng/g fortified sample processed by EN method.

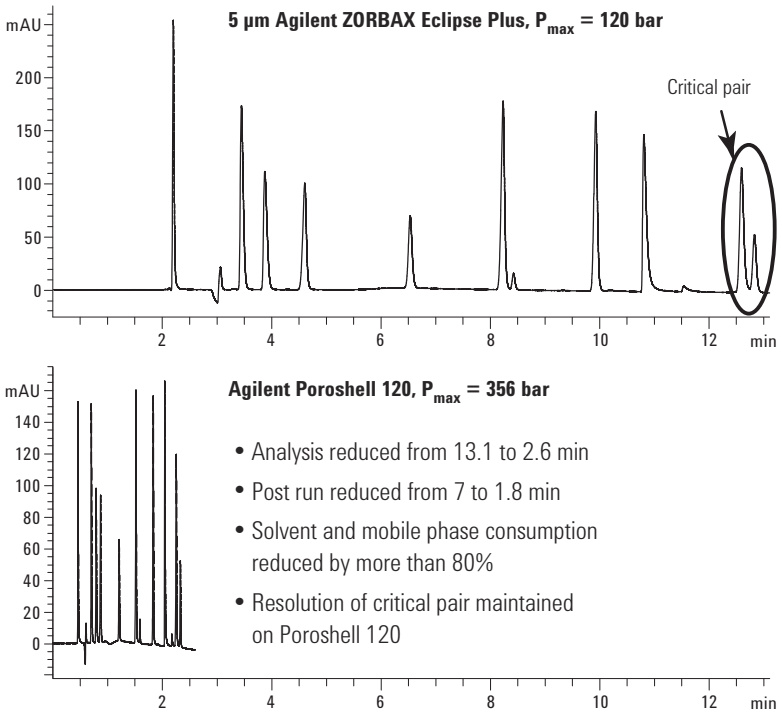
1. Acephate
2. Pymetrozine
3. Carbendazim
4. Thiabendazole
5. Imidacloprid
6. Imazalil
7. Propoxur
8. Carbaryl
9. Cyprodinil
10. Ethoprophos
11. Penconazole
12. Kresoxim-methyl

IS TPP



NEW!

An overlay of the original ZORBAX Eclipse Plus 5 µm method and Agilent Poroshell 120 method. All 11 peaks on Poroshell 120 are resolved by the time the first peak elutes on the original 5 µm ZORBAX Eclipse Plus method



- Analysis reduced from 13.1 to 2.6 min
- Post run reduced from 7 to 1.8 min
- Solvent and mobile phase consumption reduced by more than 80%
- Resolution of critical pair maintained on Poroshell 120

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	1.000 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 12.0 min
Temperature:	30 °C

Column:	**Poroshell 120 EC-C18 695975-302 3.0 x 100 mm, 2.7 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	0.851 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 2.1 min
Temperature:	30 °C

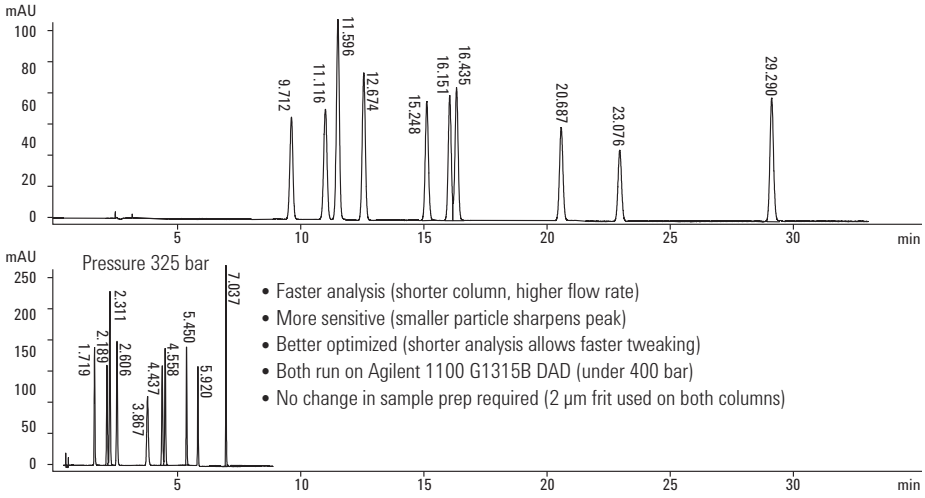
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Fast analysis of sulfa drugs

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Column:	**Poroshell 120 EC-C18 695975-902 4.6 x 100 mm, 2.7 µm**
Gradient:	Formic acid/acetonitrile
Detector:	Agilent 1100 Series LC
Sample:	Ten sulfa drugs

A separation of ten sulfa drugs scaled from an Agilent ZORBAX Eclipse Plus C18 column to an Agilent Poroshell 120 EC-C18 column showing analysis time decreased from 30 min to 8 min using a formic acid/acetonitrile gradient.



- Faster analysis (shorter column, higher flow rate)
- More sensitive (smaller particle sharpens peak)
- Better optimized (shorter analysis allows faster tweaking)
- Both run on Agilent 1100 G1315B DAD (under 400 bar)
- No change in sample prep required (2 µm frit used on both columns)

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Determination of anthocyanins in blueberries

Column: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Eclipse Plus Phenyl-Hexyl**
959758-912
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-Aq**
858700-914
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-Phenyl**
858700-912
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 5% HCOOH in H_2O
B: CH_3CN

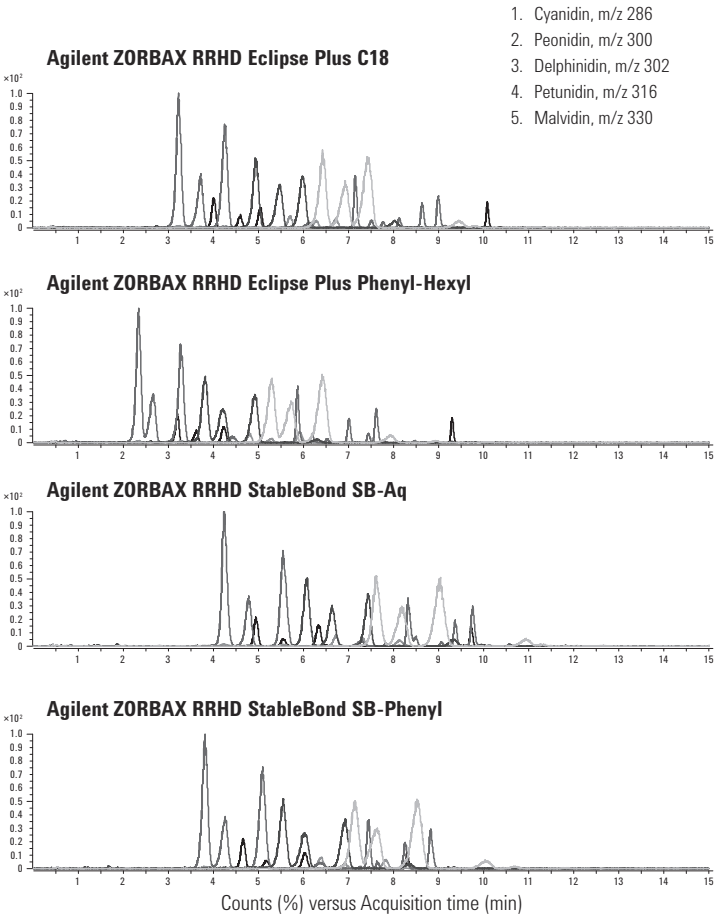
Flow Rate: 0.65 mL

Gradient: 10-50% B in 15 min

Detector: Agilent 1290 Infinity LC

MS Conditions: DAD: Sig = 525, 8 nm; Ref = Off
MS2 Scan: ESI + 200-1000
Scan time: 100 ms, 0.2 amu step
Fragmentor: 180 V
Drying gas: 10 L/min, 350 °C
Nebulizer Pressure: 50 psig
Capillary Voltage: 3500

Sample: 5 µL injection of blueberry extract



Separation of Azo Dyes

Column: **Eclipse Plus Phenyl Hexyl**
959996-912
4.6 x 100 mm, 5 µm

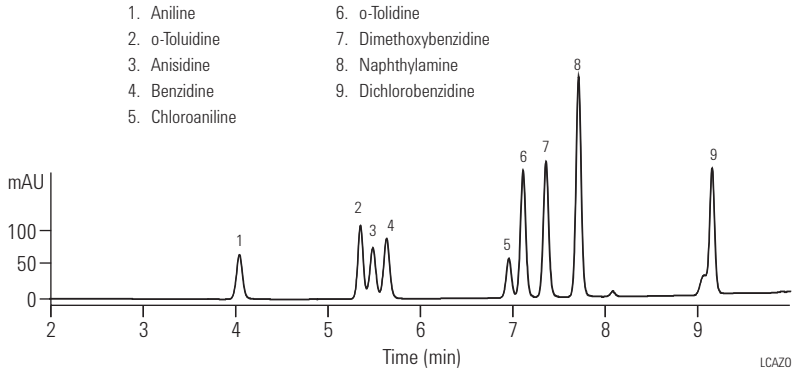
Mobile Phase: A: 10 mM Ammonium Acetate, pH 4.7
B: MeOH

Flow Rate: 1.5 mL/min

Gradient:

Time (Min):	%B:
0	25
5	50

Detector: UV, 254 nm



Anthocyanins from blueberries: High-efficiency high-speed separation

Column A: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

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

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

Mobile Phase: A: 3% Phosphoric acid
B: 100% MeOH

Flow Rate: 1.0 mL/min

Gradient: As shown

Temperature: 30 °C

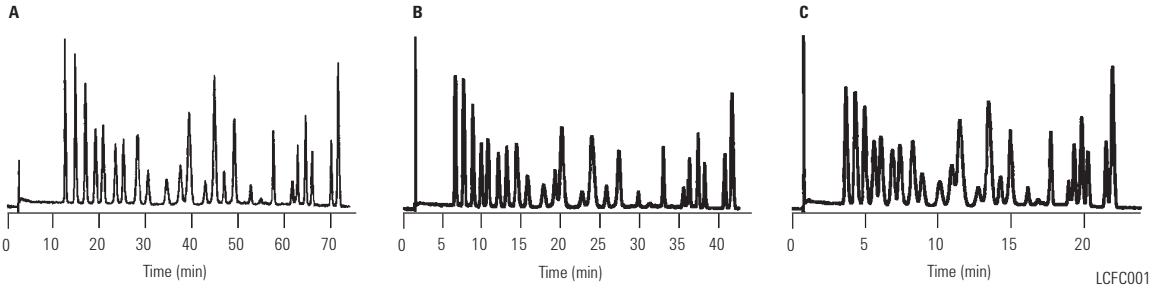
Detector: UV, 525 nm

Sample: Natural anthocyanins

Time	Percent B
0 min	23% B
35 min	26% B
97 min	60% B

Time	Percent B
0 min	23% B
21 min	26% B
58.2 min	60% B

Time	Percent B
0 min	23% B
10.5 min	26% B
29.1 min	60% B



Aromatics II

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

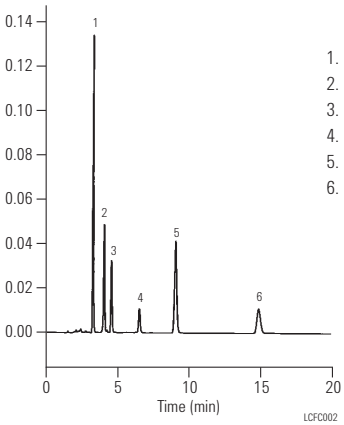
Mobile Phase: H_2O: MeOH, 40:60

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Aromatic Sample



1. Acetophenone
2. Cinnamaldehyde
3. Eugenol
4. Cinnamaldehyde Impurity
5. Ethyl cinnamate
6. p-Cymene

Aspartame: Metabolites and applications

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

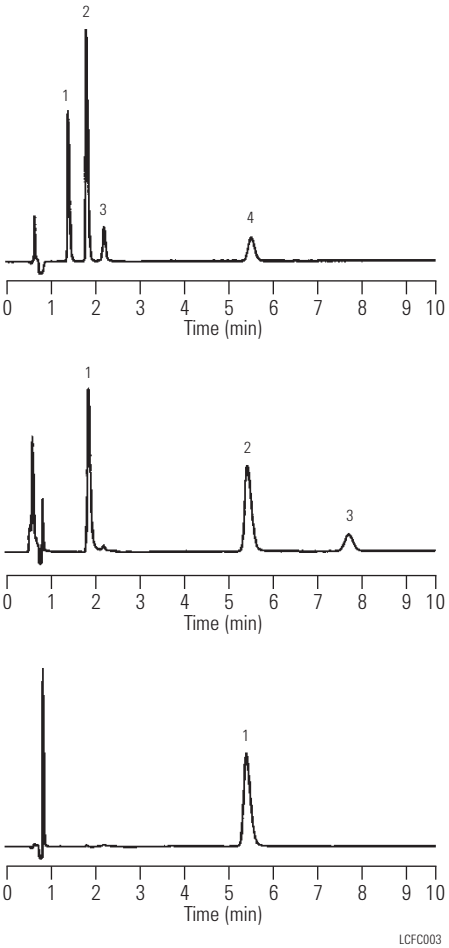
Mobile Phase: 85/15, 0.1% TFA/ACN

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 210 nm

Sample: Aspartame



Aspartame and Its Metabolites

1. Phenylalanine
2. 5-benzyl-3,6-dioxo-2-piperazineacetic acid
3. Aspartic acid-phenylalanine dipeptide
4. Aspartame

Diet Coke

1. Caffeine
2. Aspartame
3. Unknown

Equal Sweetener

1. Aspartame

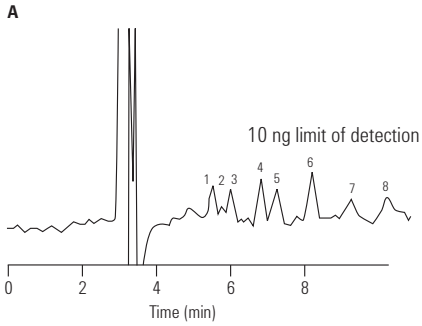
Carbohydrates: Carbohydrate standards

Column: **ZORBAX Carbohydrate Analysis**
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 63% CH_3CN/H_2O
Flow Rate: 0.5 mL/min

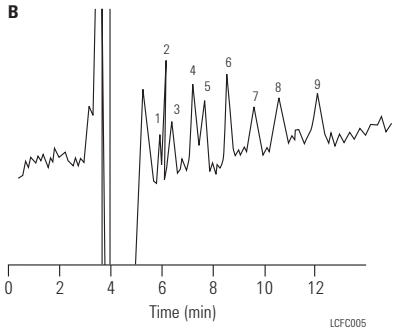
Detector: Agilent RID
Sample: Carbohydrate standard:
A: 25 ng/ L, 1 µL injected
B: 500 pg/ L, 50 µL injected

Carbohydrates: Separation showing high sensitivity



1. Ribose
2. Rhamnose
3. Xylose
4. Fructose
5. Glucose
6. Sucrose
7. Maltose
8. Lactose
9. Raffinose

Sensitivity of high injection volume (50 µL)



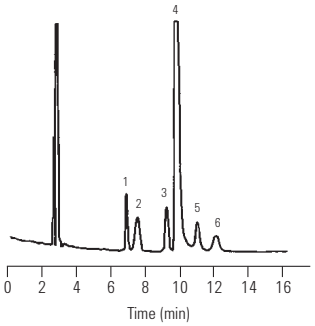
Carbohydrates: Effect of mobile phase strength

Column: **ZORBAX NH2**
880952-708
4.6 x 250 mm, 5 µm

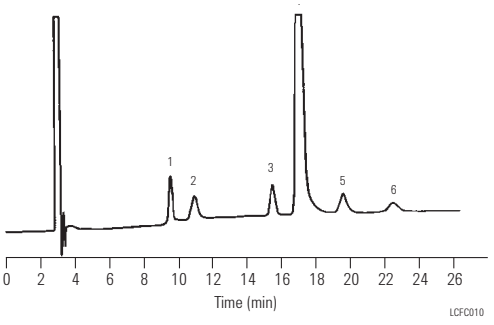
Mobile Phase: ACN/Water, as indicated
Flow Rate: 1.0 mL/min

Temperature: Ambient
Detector: RI
Sample: Mono- and Disaccarides

ACN/H_2O: 70/30



ACN/H_2O: 75/25



1. Fructose
2. Glucose
3. Saccharose
4. Palatinose
5. Trehalulose
6. Isomaltose

Carbohydrates in colas

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

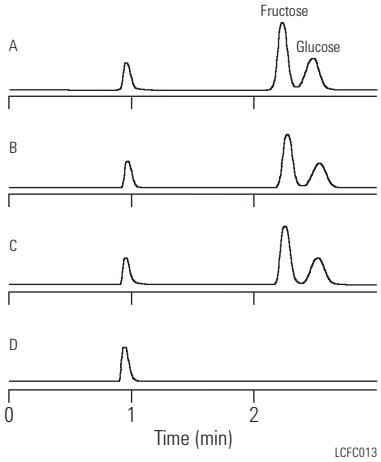
Mobile Phase: 75% ACN:25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: No dilution
A: COLA, Fountain
B: COLA, Can, Brand A
C: COLA, Brand B
D: COLA, Brand B, diet



Carbohydrates: Sugar alcohols

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

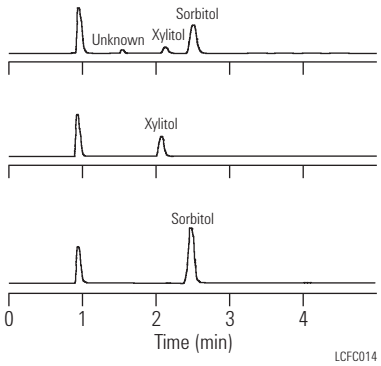
Mobile Phase: 75% ACN:25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Chewing gum, sugar-free



Carbohydrates in juices

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

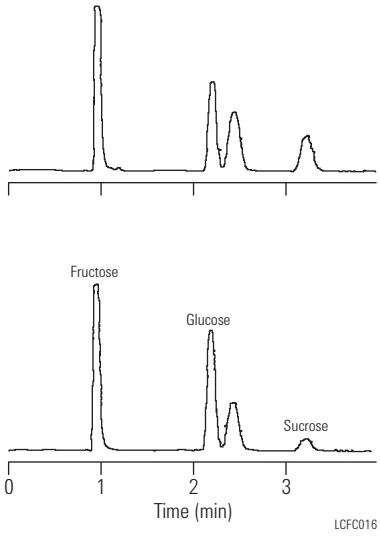
Mobile Phase: 75% ACN/25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Diluted to 0.1X in 50:50 ACN:H_2O



Apple Drink
36.8% Fructose
24.9% Sucrose
38.3% Glucose

Apple Juice
58.7% Fructose
9.9% Sucrose
33.4% Glucose

Carbohydrates in milk

Column: **ZORBAX Carbohydrate Analysis**
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 75% ACN/25% H_2O

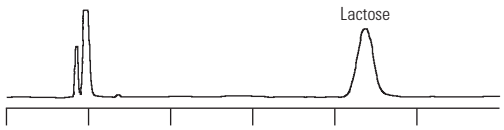
Flow Rate: 2.0 mL/min

Temperature: 30 °C

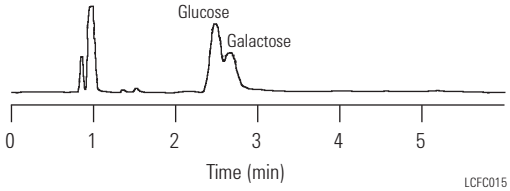
Detector: RID

Sample: Partitioned between CH_3Cl_2: H_2O

Milk (2%)



100% Lactose-Free Milk



Flavoring agents

Column: **ZORBAX SB-Phenyl**
860975-912
2.1 x 50 mm, 5 µm

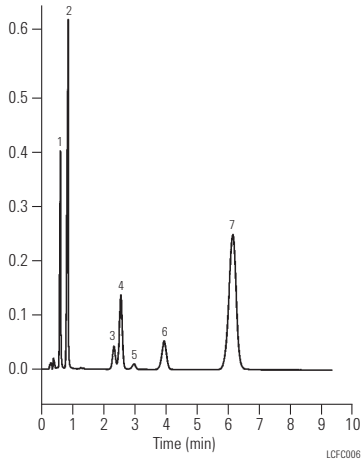
Mobile Phase: 0.3% TFA: ACN, 65:35

Flow Rate: 0.3 mL /min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Cool mint Listerine sample



1. Unknown
2. Benzoic acid
3. Methyl salicylate
4. Carvone
5. Unknown
6. Thymol
7. Anethole

Food colors, FD&C

Column: **ZORBAX Eclipse XDB-C18**
935967-902
4.6 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA, pH to 4.4 with TEA, B: MeOH

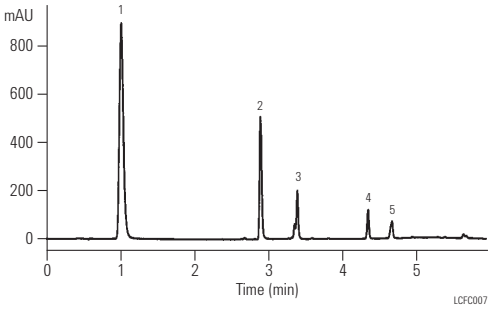
Flow Rate: 1.0 mL/min

Gradient: 17 to 100% B/4 min

Temperature: Ambient

Detector: UV, 254 nm

1. Yellow #5	$C_{16}H_9N_4Na_3O_9S_2$	MW=534
2. Red #40	$C_{18}H_{14}N_2Na_2O_8S_2$	MW=496
3. Blue #1	$C_{37}H_{34}N_2Na_2O_9S_3$	MW=760
4. Propylparaben	$C_{10}H_{12}O_3$	MW=180
5. Red #3	$C_{20}H_{14}Na_2O_5$	MW=878



Neutraceuticals: Extract from green tea

Column: **ZORBAX SB-C8**
863953-906
4.6 x 150 mm, 3.5 µm

Mobile Phase: 75% 0.1% Trifluoroacetic acid: 25% Methanol

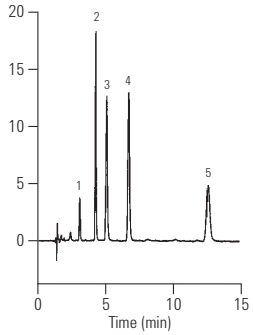
Injection: 1 mL/min

Temperature: 40 °C

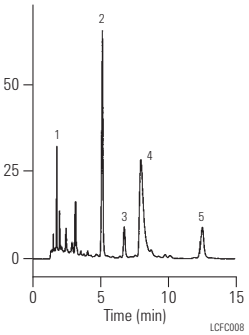
Detector: UV, 280 nm

Sample: Green tea extract, 5 µL

Catechin Mixture



Green Tea Extract



LCFC008

1. Epagallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

Tocopherols by LC/MS with APPI

Column: **Eclipse XDB-C18**
993967-302
3.0 x 150 mm, 5 µm

Mobile Phase: 97% MeOH: 3% 10 mM CH_3COONH_4

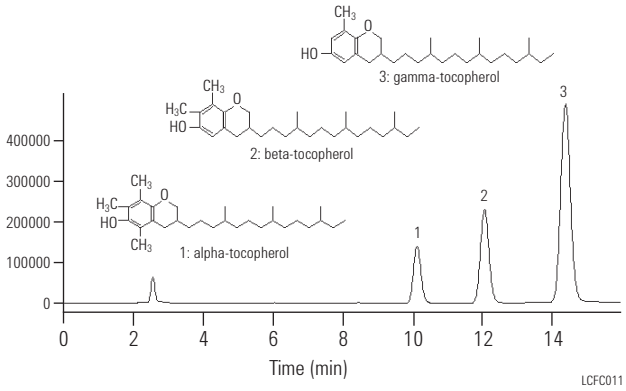
Flow Rate: 0.5 mL/min

Temperature: 40 °C

MS Conditions:

MS:	Agilent 1100MSD SL
Ionization:	APPI (Positive)
Scan range:	m/z 100-500
Vcap:	1500 V
SIM ion:	base peak
Drying gas:	7 L/min at 350 °C
Nebulizer gas:	60 psi
Vaporizer temp:	350 °C
Fragmentor:	140 V
EM gain:	4

Sample Volume: 10 µL



LCFC011

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Sugars in plain and milk chocolate

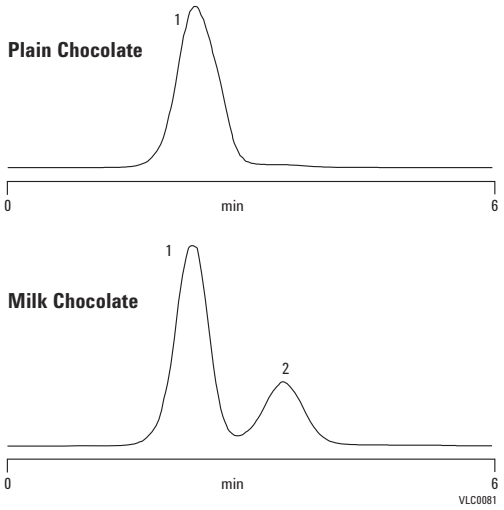
Column: **Hi-Plex Pb**
PL1170-6820
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 80 °C

Detector: RI



1. Sucrose
2. Lactose

Sugars

Column: **Hi-Plex K**
PL1170-6860
7.7 x 300 mm, 8 µm

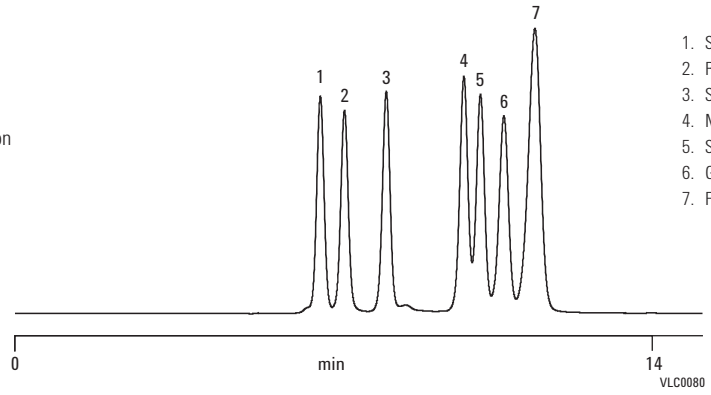
Sample: Sugars mixture (all 10 mg/mL), 20 µL injection

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: 356-LC RI



1. Stachyose
2. Raffinose
3. Sucrose
4. Mannitol
5. Sorbitol
6. Glucose
7. Fructose

Parabens: High speed separation

Column: **ZORBAX SB-C18 Rapid Resolution Cartridge**
833975-902
4.6 x 30 mm, 3.5 µm

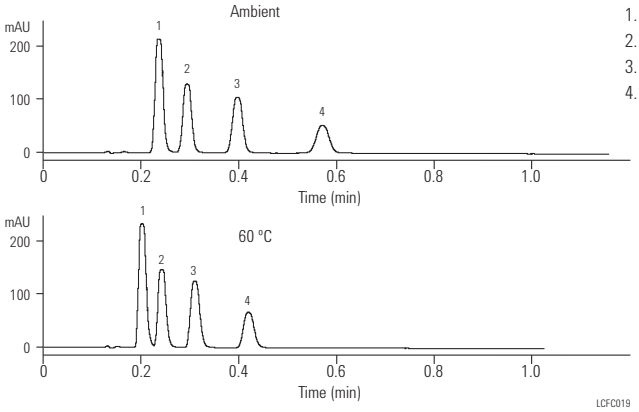
Mobile Phase: 0.1% H_3PO_4:ACN, (50:50)

Flow Rate: 2 mL/min

Temperature: Top: ambient, bottom: 60 °C

Detector: UV, 254 nm with standard flow cell (13 µL)

Sample: Parabens, 1 µL



1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben

Separation of vitamin D2/D3

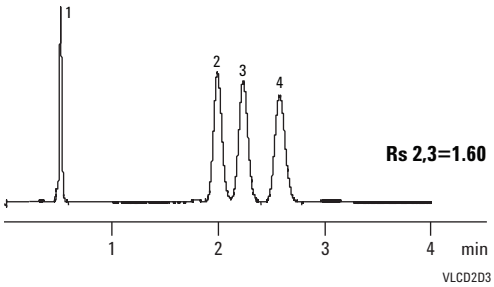
Column: **Eclipse PAH**
959941-918
4.6 x 50 mm, 1.8 µm

Mobile Phase: 92% MeOH, 8% water

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: 325 nm for VA/280 nm for VD and VE



1. Vitamin A
2. Vitamin D2
3. Vitamin D3
4. Vitamin E (a-VE)

Fat-soluble vitamins on ZORBAX Eclipse XDB-C8

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

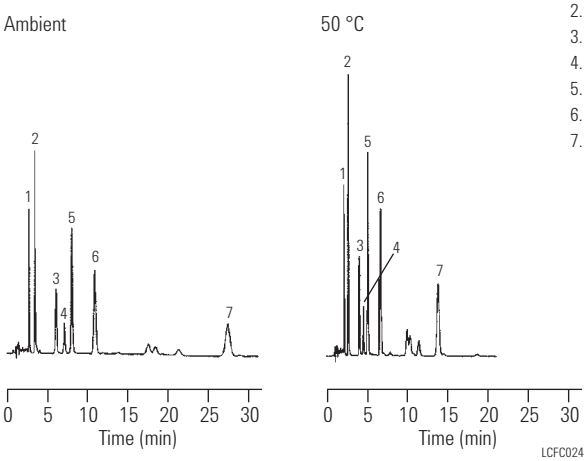
Mobile Phase: 5/95 Water/MeOH

Flow Rate: 1.0 mL/min

Temperature: A: Ambient
B: 50 °C

Detector: UV, 280 nm

Sample: Fat-soluble vitamins



1. Retinol
2. Retinol acetate
3. Vitamin D3
4. γ-Tocopherol
5. α-Tocopherol
6. Tocopherol acetate
7. Retinol palmitate

Water-soluble vitamins

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

Mobile Phase: A: 50 mM Sodium Phosphate, pH 2.5/MeOH (90/10)
B: 50 mM Sodium Phosphate, pH 2.5/MeOH (10/90)

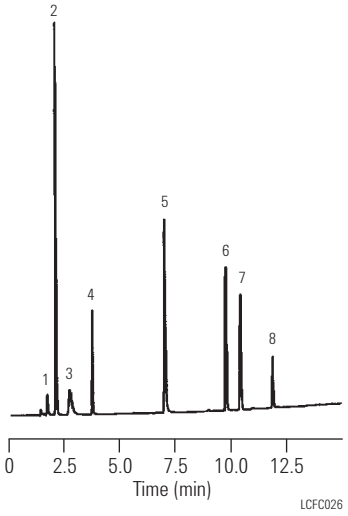
Flow Rate: 1.0 mL/min

Gradient: 0-70% B in 18 min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. B_1-Thiamine
2. Vitamin C
3. B_3-Niacin
4. B_6-Pyridoxine
5. Pantothenic acid
6. Folic acid
7. B_{12}-Cyanocobalamin
8. B_2-Riboflavin

Water-soluble vitamins: High speed separation using ion-pairing

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

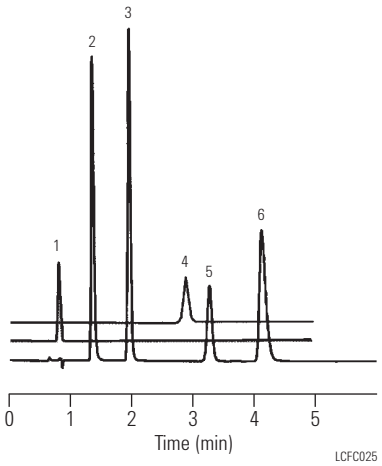
Mobile Phase: 10 mM Hexane Sulfonate with 0.1% Phosphoric Acid: MeOH (74:26)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. Vitamin C
2. B_3-Niacin
3. B_6-Pyridoxine
4. Folic acid
5. B_2-Riboflavin
6. B_1-Thiamine

Water-soluble vitamins using the USP 23 method

Column: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

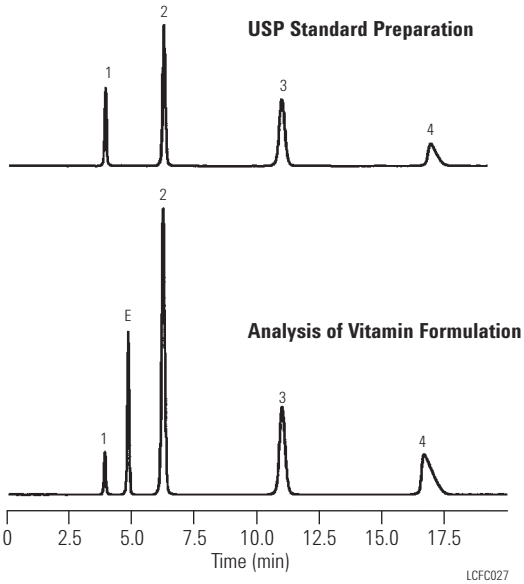
Mobile Phase: 7.2 mM Hexane Sulfonate/MeOH/Acetic Acid (73/27/1) (ratio to 101)

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 280 nm

Sample: Water-soluble vitamins



1. B_3-Niacin
2. B_6-Pyridoxine
3. B_2-Riboflavin
4. B_1-Thiamine
E. Excipent

Water-soluble B vitamins separated on ZORBAX SB-Aq

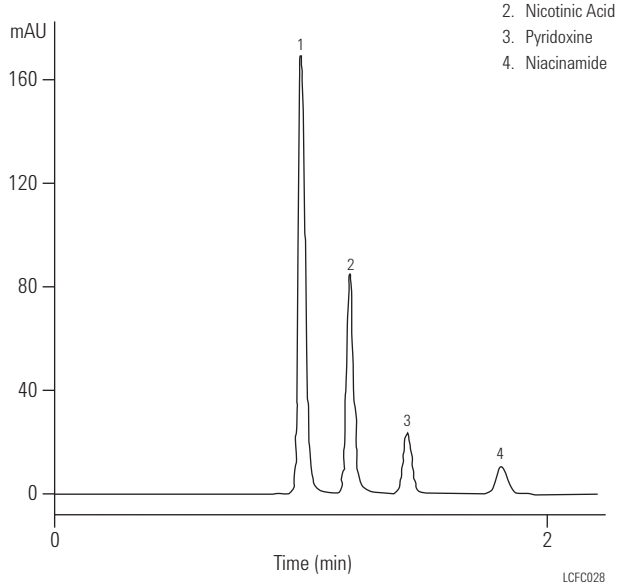
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 5% MeOH/95% water (0.1% TFA)

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



1. Thiamine
2. Nicotinic Acid
3. Pyridoxine
4. Niacinamide

Sunscreen ingredients: Perform conventional, fast and ultra-fast separations on the same column family

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

Column B: **Eclipse XDB-C18**
961967-902
4.6 x 100 mm, 3.5 µm
4 µL inj

Column C: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm
2 µL inj

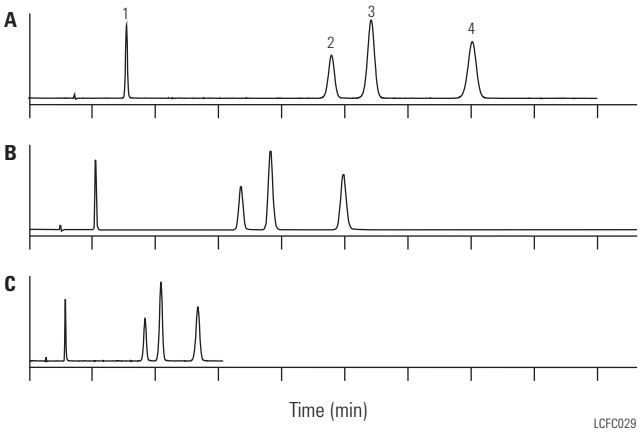
Mobile Phase: A: 15% water
B: 85% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Sunscreens



1. 2-hydroxy-4-methoxybenzophenone
2. Padimate O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate

Fast vitamin E analysis on Rapid Resolution HT

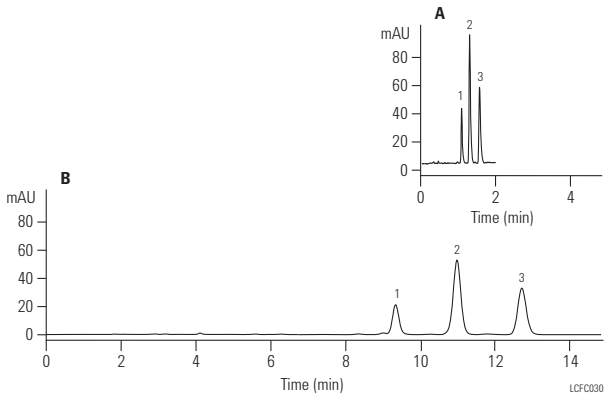
Column A: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm

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

Mobile Phase: A: 5% water
B: 95% MeOH

Flow Rate: 3 mL/min, 1 mL/min

Temperature: Ambient



1. α-tocopherol
2. β-tocopherol
3. γ-tocopherol

Theobromine in beverages

Column: **ZORBAX SB-C18**
827975-902
4.6 x 50 mm, 1.8 µm

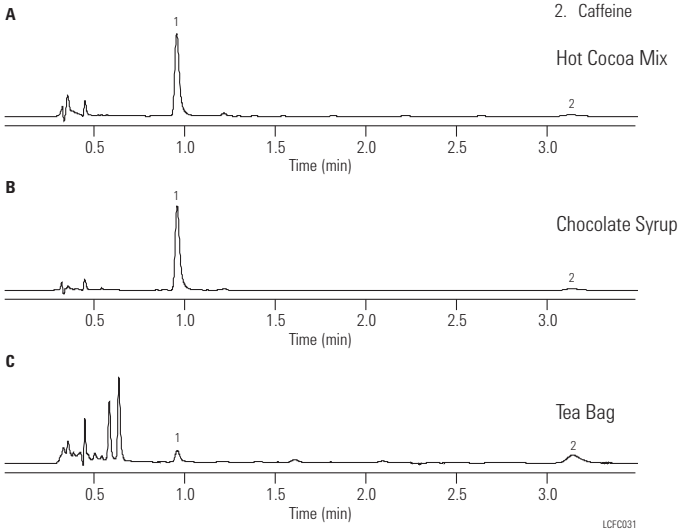
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm, flow cell 2 µL,
3 mm flow path

Sample: Theobromine



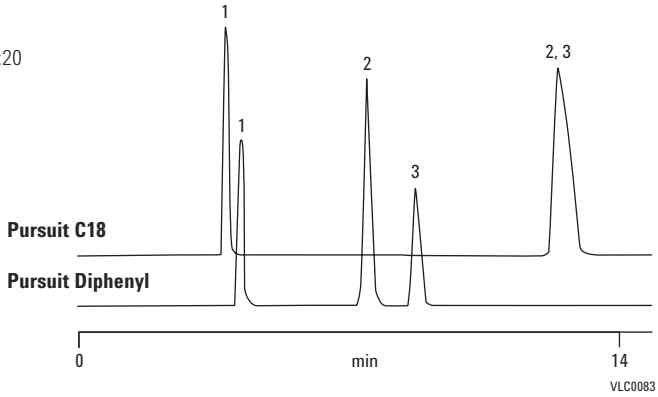
1. Theobromine
2. Caffeine

Benzoic acid/sorbic acid

Mobile Phase: 0.1% formic acid in water:
0.1% formic acid in MeCN, 80:20

Flow Rate: 0.7 mL/min

Detector: UV, 254 nm



1. p-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid

Quantification and qualification of vitamin C and citric acid in fresh grapefruit juice

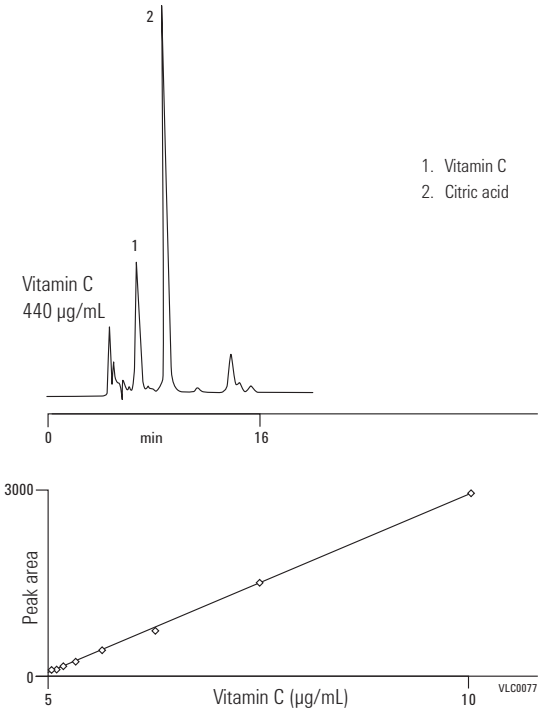
Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

Sample: Diluted 1:50 in eluent

Mobile Phase: 0.2M NaH_2PO_4, pH 2.14

Flow Rate: 0.5 mL/min

Detector: UV, 220 nm



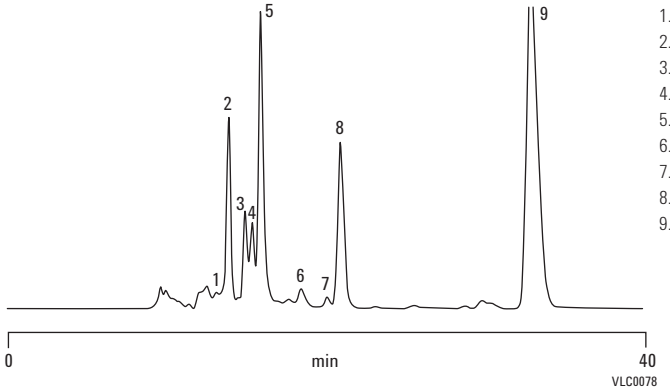
1. Vitamin C
2. Citric acid

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Rose wine

Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: 0.004M H_2SO_4
Flow Rate: 0.4 mL/min
Pressure: 13 bar
Temperature: 75 °C
Detector: RI

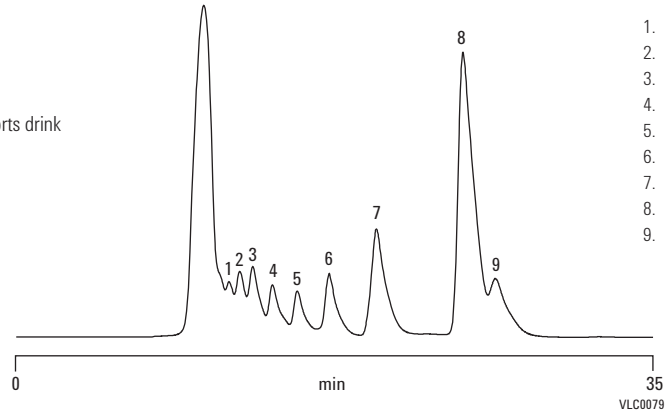


1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Succinic acid
7. Lactic acid
8. Glycerol
9. Ethanol

Sports drink

Column: **Hi-Plex Na**
PL1171-6140
7.7 x 300 mm, 10 µm

Sample: High energy orange flavor non-carbonated sports drink
Mobile Phase: Water
Flow Rate: 0.3 mL/min
Temperature: 80 °C
Detector: RI

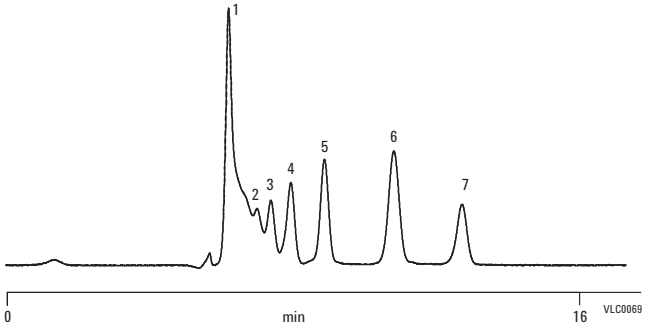


1. Dp8
2. Dp7
3. Dp6
4. Dp5
5. Dp4
6. Dp3
7. Dp2
8. Dp1 (Glucose)
9. Fructose

Oligosaccharides

Column: **Hi-Plex Ca (Duo)**
PL1F70-6850
6.5 x 300 mm, 8 µm

Mobile Phase: DI water
Flow Rate: 0.5 mL/min
Temperature: 90 °C
Detector: RI



1. Higher MW sugars
2. DP5
3. DP4
4. DP3
5. DP2
6. DP1
7. Fructose

Pharmaceutical Applications

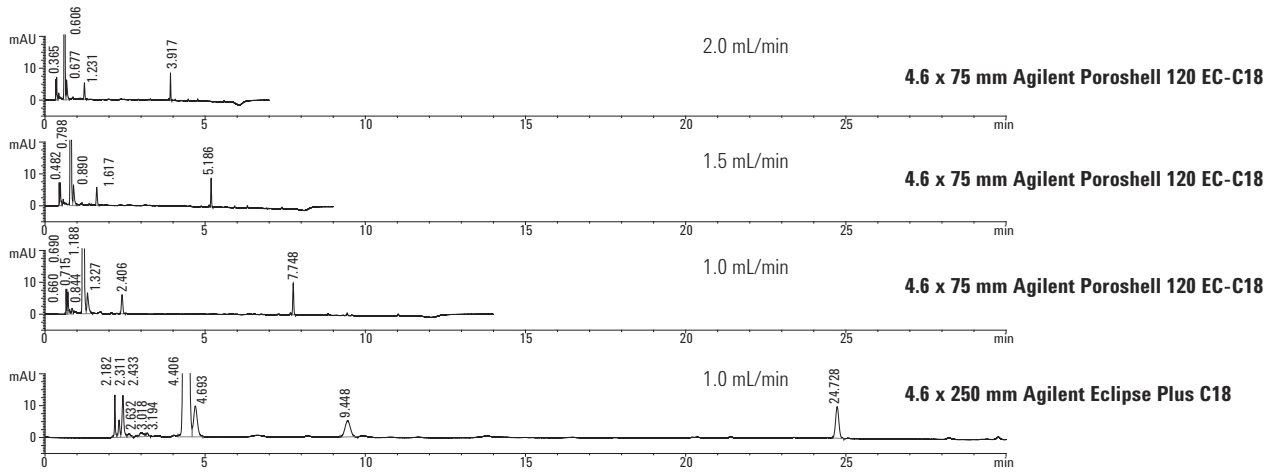
NEW!

Fast analysis of cefepime and related impurities

Column: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Column: **Eclipse Plus C18**
959990-902
4.6 x 250 mm, 5 µm

Detector: Agilent 1200 Infinity Series



NEW!

Naproxen analysis

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

Column B: **Poroshell 120 EC-C18**
699975-902
4.6 x 50 mm, 2.7 µm

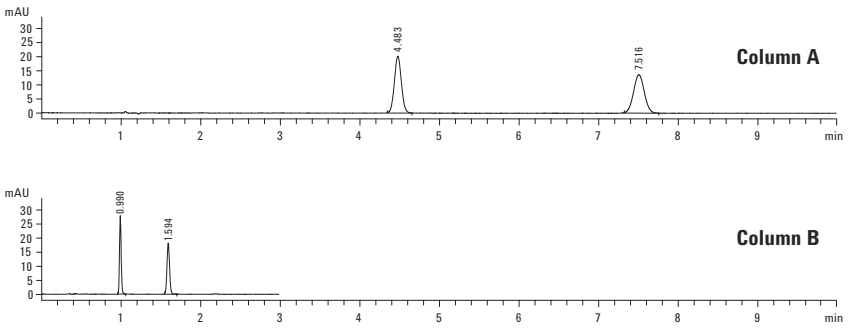
Mobile Phase: 50:49:1 MeCN:H_2O:Glacial acetic acid

Flow Rate: 1.2 mL/min

Injection: Column A: 20 µL
Column B: 6.7 µL

Injection: Naproxen

Method requirement N > 4000 Rs better than 11.5



4-fold reduction in analysis time for this method when transferring to Poroshell 120.

NEW!

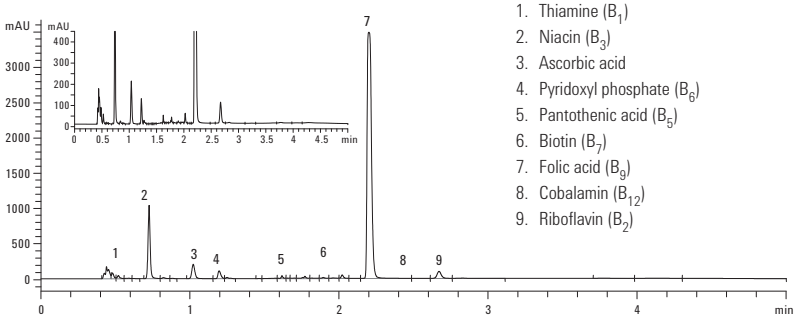
Analysis of water soluble vitamins in multivitamin tablets

Column: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Flow Rate: 1.5 mL/min

Gradient: 0 min-1% B, 0.5 min-12% B, 0.52 min-30% B, 3.5 min-30% B, 4.5 min-1% B

Injection: 5 µL



1. Thiamine (B_1)
2. Niacin (B_3)
3. Ascorbic acid
4. Pyridoxyl phosphate (B_6)
5. Pantothenic acid (B_5)
6. Biotin (B_7)
7. Folic acid (B_9)
8. Cobalamin (B_{12})
9. Riboflavin (B_2)

NEW!

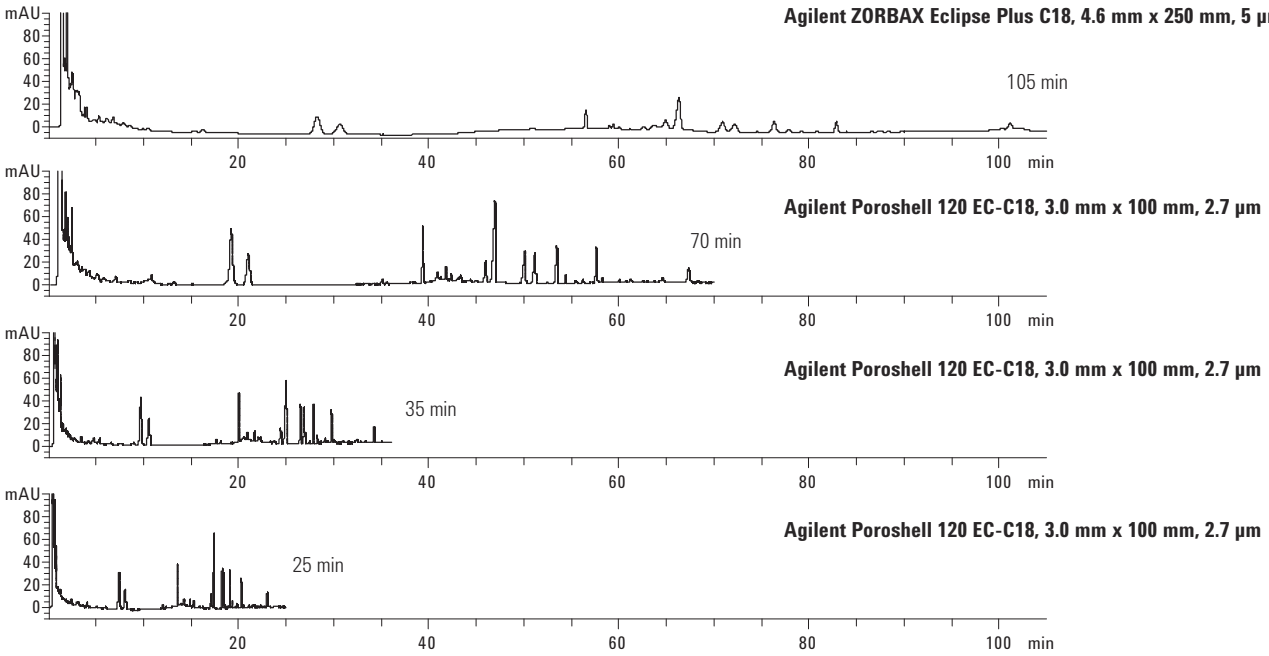
Fast method for ginseng analyses scaled from a traditional method

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

Column: **Poroshell 120 EC-C18**
695975-302
3.0 x 100 mm, 2.7 µm

Detector: 1200 Infinity Series

Sample: Ginsenoside



Agilent ZORBAX Eclipse Plus C18, 4.6 mm x 250 mm, 5 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

NEW!

Separation of 8 steroids

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

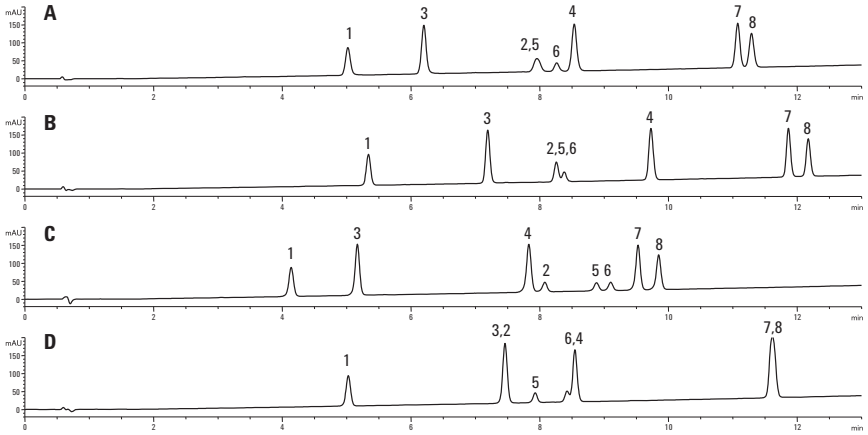
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Mobile Phase: 0.1% formic acid in both water and MeOH

Flow Rate: 0.4 mL/min, 25 ºC, 2.1 x 100 mm 40 ºC

Gradient: 40-80% MeOH in 14 min

1. Hydrocortisone
2. β-Estradiol
3. Androstadiene 3,17 dione
4. Testosterone
5. Ethynylestradiol
6. Estrone
7. Norethindrone acetate
8. Progesterone



NEW!

Mixture of beta blockers

Column A: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column D: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

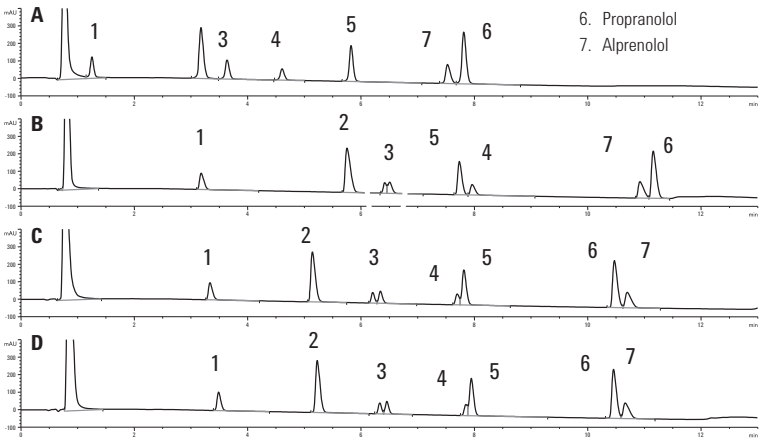
Mobile Phase: 10 mM pH 3.8 NH_4HCO_2, methanol

Flow Rate: 0.35 mL/min

Gradient: 90% B to 30% B over 12 min

* Nadolol is isobaric and elutes as two peaks.

1. Atenolol
2. Pindolol
3. Nadolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol



NEW!

Several ZORBAX RRHD 1.8 µm selectivities facilitate method development

Column: **ZORBAX RRHD Eclipse Plus C18**
959758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Eclipse XDB-C18**
981758-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD SB-C18**
858700-902
2.1 x 100 mm, 1.8 µm

Column: **ZORBAX RRHD Extend-C18**
758700-902
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: H_2O
B: CH_3CN, each with 0.1% HCOOH

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

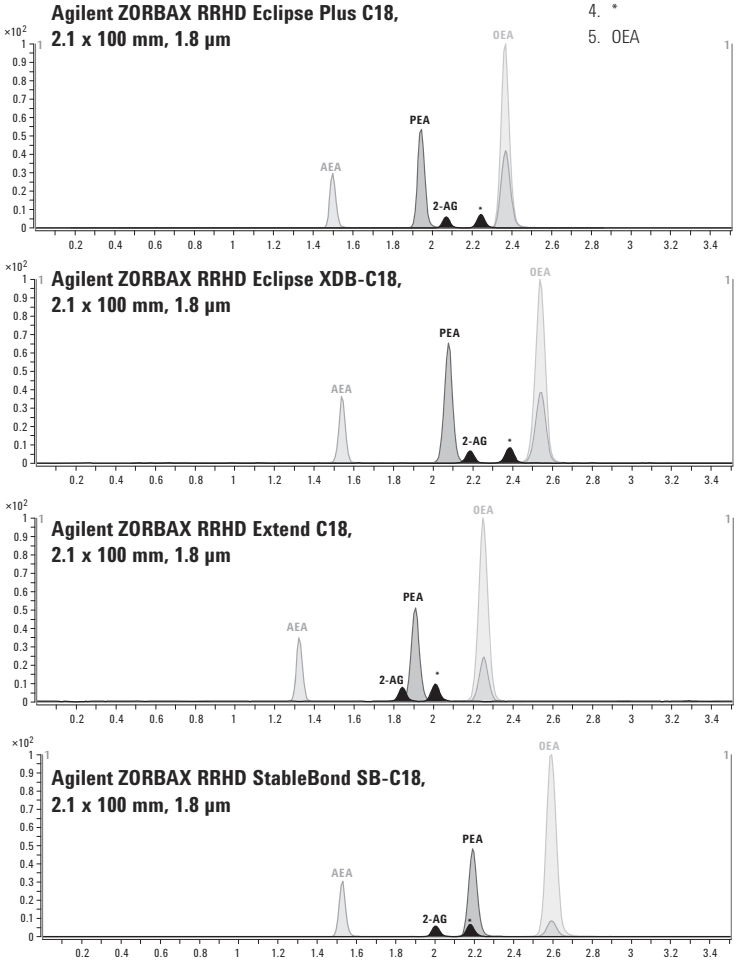
MS Conditions: TCC: 30 °C
MS Source: Electrospray AP-ESI
Drying-gas temperature and flow: 325 °C, 12 L/min
Nebulizer gas pressure: 35 psi
Capillary voltage: 3000 V

Sample: Four endocannabinoid fatty amides:
Arachidonoylglycerol (AEA)
2-Arachidonoylglycerol (2-AG)
Palmitoylethanolamide (PEA)
Oleoylethanolamide (OEA)

*** The second black peak is an impurity, believed to be 1,3-arachidonolyglycerol, a rearrangement of 2-AG**

The selectivity of four Agilent ZORBAX RRHD C18 columns is compared using a method for endocannabinoids.

1. AEA
2. PEA
3. 2-AG
4. *
5. OEA



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Fast analysis 11 common compounds found in analgesics

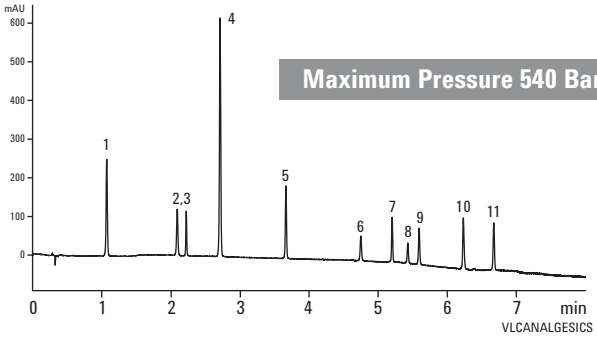
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: A : Water + 0.1% formic acid
B: ACN

Flow Rate: 3.5 mL/min

Temperature: 40 °C

Detector: DAD 254 nm



Maximum Pressure 540 Bar

1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusinal
11. Diclofenac

Faster analysis of USP Method for simvastatin tablet

Column A: **Eclipse Plus C18**
959990-902
4.6 x 250 mm, 5 µm

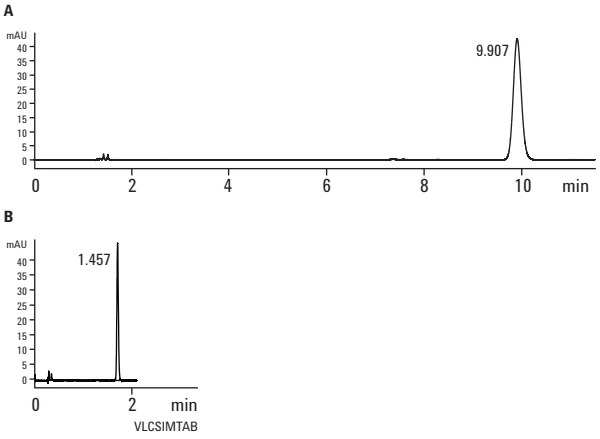
Column B: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Mobile Phase: 65% CH_3CN,
35% 3.9 g/L NaH_2PO_4 (pH 4.5)

Flow Rate: 1.5 mL/min for 5 µm column
2.8 mL/min for 2.7 µm Poroshell 120 column

Temperature: 45 °C

Detector: DAD Sig = 238, 8
Ref = 360, 100 nm



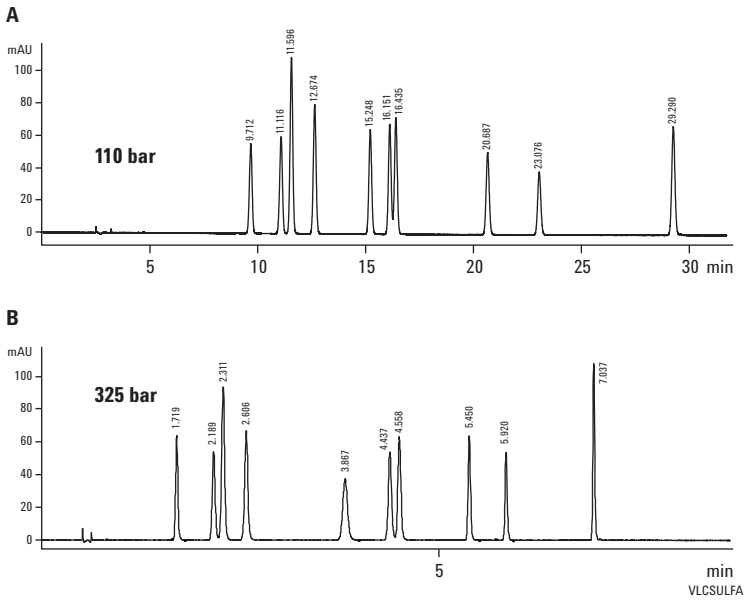
	USP Requirement	5 µm (1.5 mL/min)	2.7 µm (2.8 mL/min)
T_R	N/A	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

Faster separation of sulfa drugs

		Time	%B
Column A:	**Eclipse Plus C18**	0	8
	959990-902	33	33
	4.6 x 250 mm, 5 µm	35	33
Column B:	**Poroshell 120 EC-C18**	**Time**	**%B**
	695975-902	0	8
	4.6 x 100 mm, 2.7 µm	12	33
		13.2	33

Mobile Phase: A: 0.1% formic acid in Water
B: 0.1% formic acid in ACN

Flow Rate: 1 mL/min



Separation of pharmaceutical cardiac drugs

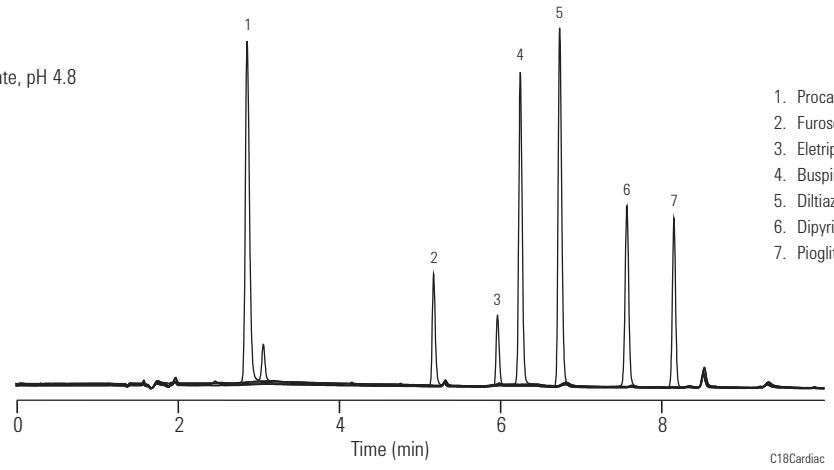
Column: **Eclipse Plus C18**
959996-902
4.6 x 100 mm, 5 µm

Mobile Phase: A: 20 mM Ammonium Acetate, pH 4.8
B: ACN

Flow Rate: 1 mL/min

Gradient: 10-90% in 10 min

Detector: UV, 254 nm



1. Procainamide
2. Furosemide
3. Eletriptan
4. Buspirone
5. Diltiazen
6. Dipyridamole
7. Pioglitazone

Fast and ultra-fast analysis of basic compounds

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

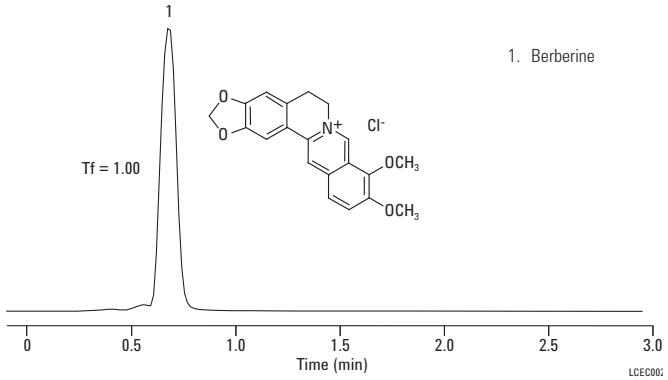
Mobile Phase: A: 50% 8 mM K_2HPO_4, pH 7
B: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Berberine, 0.4 mg/mL, 2 µL



1. Berberine

Xanthines: Higher resolution, same selectivity with RRHT

Column A: **ZORBAX SB-C18**
846975-902
4.6 x 50 mm, 5 µm

Column B: **ZORBAX SB-C18**
827975-902
4.6 x 50 mm, 1.8 µm

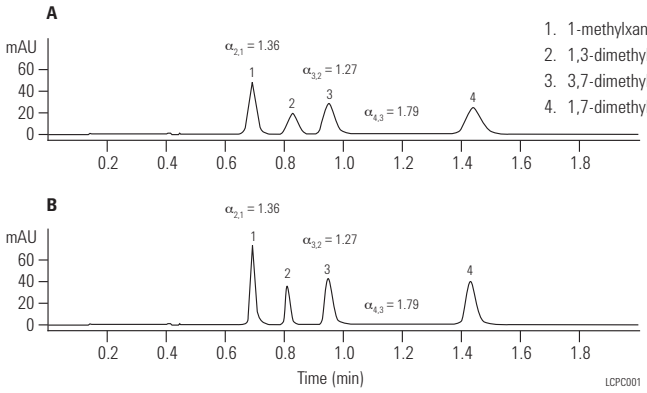
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Xanthines



1. 1-methylxanthine
2. 1,3-dimethyluric acid
3. 3,7-dimethylxanthine (theobromine)
4. 1,7-dimethylxanthine

Antihistamines: Fast separations on RRHT Extend-C18

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

Column B: **ZORBAX Extend-C18**
727975-902
4.6 x 50 mm, 1.8 µm

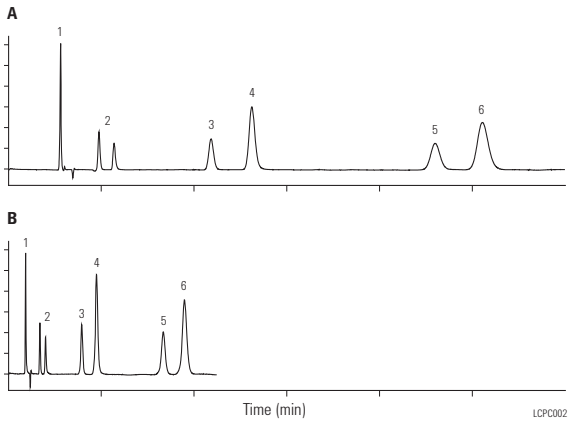
Mobile Phase: A: 30% 50 mM pyrrolidine buffer
B: 70% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antihistamines



1. Scopolamine
2. Pseudoephedrine
3. Doxylamine
4. Chlorpheniramine
5. Diphenhydramine
6. Triprolidine

Ibuprofen:
Optimizing selectivity with RRHT Columns

Column A: **SB-C8**
827975-906
4.6 x 50 mm, 1.8 µm

Column B: **Eclipse XDB-C8**
927975-906
4.6 x 50 mm, 1.8 µm

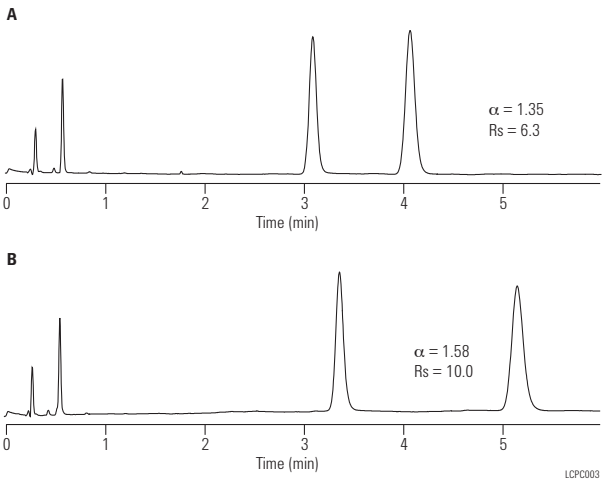
Mobile Phase: A: 63% water
B: 37% acetonitrile + 1.8 mL H_3PO_4

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Ibuprofen oral suspension



1. Benzophenone
2. Ibuprofen

Analgesics

Column: **Pursuit XRs Diphenyl**
A6020150X046
4.6 x 150 mm, 5 µm

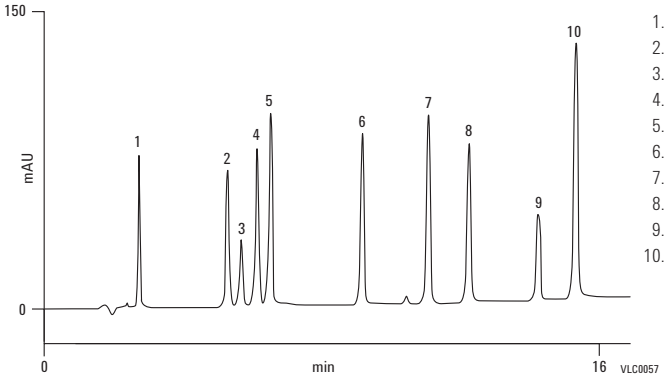
Mobile Phase: A: Water+0.1% HCOOH
B: MeCN+0.1% HCCOH

Gradient: 25-80% B in 20 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. Acetaminophen
2. Acetanilide
3. Salicylic acid
4. Acetylsalicylic acid
5. Phenacetin
6. Carbamazepine
7. Tolmetin
8. Naproxen
9. Ibuprofen
10. Diclofenac

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Anesthetics, local: Bonded phase selectivity

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

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

Column C: **ZORBAX SB-C3**
883975-909
4.6 x 150 mm, 5 µm

Column D: **ZORBAX SB-Phenyl**
883975-912
4.6 x 150 mm, 5 µm

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

Mobile Phase: A: 50 mM NaH_2PO_4 pH 2.5 in 95% H_2O/5% ACN
B: 50 mM NaH_2PO_4 pH 2.5 in 47% H_2O/53% ACN

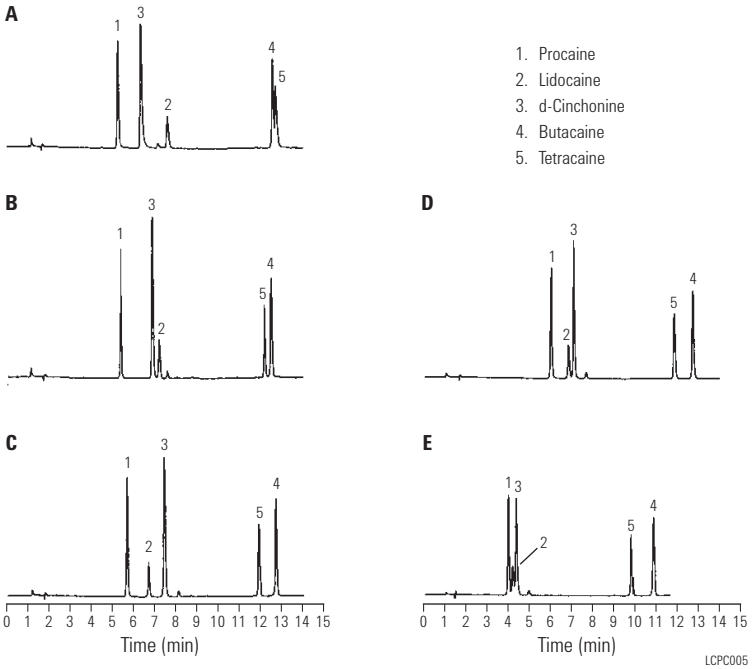
Flow Rate: 1.5 mL/min

Gradient: 0-100% B in 18.8 min

Temperature: 26 °C

Detector: UV, 254 nm

Sample: 10 µL, 10 ug/mL



1. Procaine
2. Lidocaine
3. d-Cinchonine
4. Butacaine
5. Tetracaine

Local anesthetics

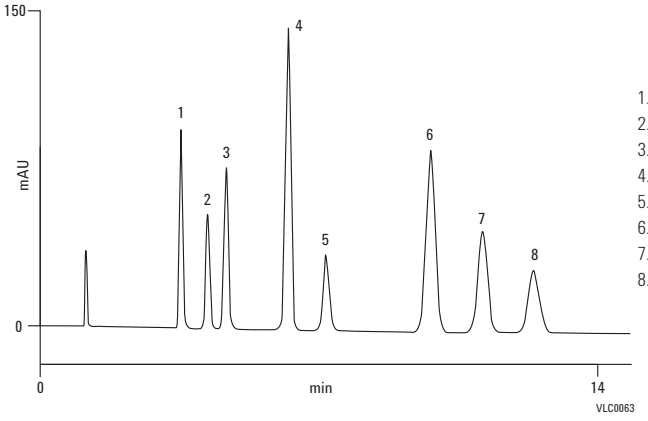
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: 65:35 MeOH:5 mM NH_4CO_3, pH 10

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 210 nm



1. Benzocaine
2. Procaine
3. Chlorocaine
4. Mepivacaine
5. 4-Hydroxyropivacaine
6. Cocaine
7. Lidocaine
8. Ropivacaine

Antibiotics: High speed separation

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

Mobile Phase: 8.0% acetonitrile/92% 0.1% aqueous TFA

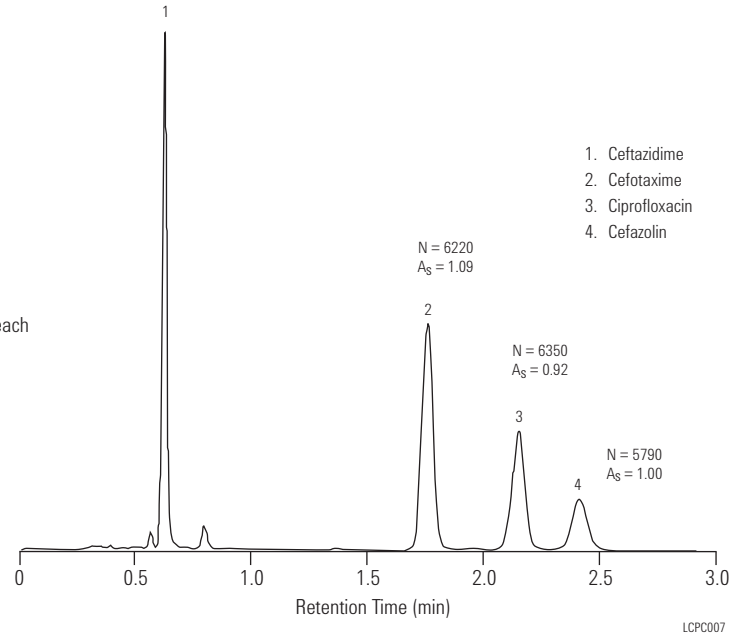
Flow Rate: 3.0 mL/min

Gradient: 45-70% B in 35 min

Temperature: 60 °C

Detector: UV, 260 nm

Sample: 1 µL containing 0.40, 0.36, 0.10 and 0.37 µg each of 1-4 resp.



Antibiotics: Lincomycin and Clindamycin by LC-APCI-MS LC-TIC

Column: **ZORBAX SB-C18 cartridge**
823700-902
2.1 x 30 mm, 1.8 µm

Mobile Phase: Gradient: 15-50% B in 1 min, hold for 1.5 min,
A: 0.2% formic acid pH 2.8
B: ACN + 0.2% formic acid

Flow Rate: 0.5 mL/min

Gradient: Post time: 1.5 min

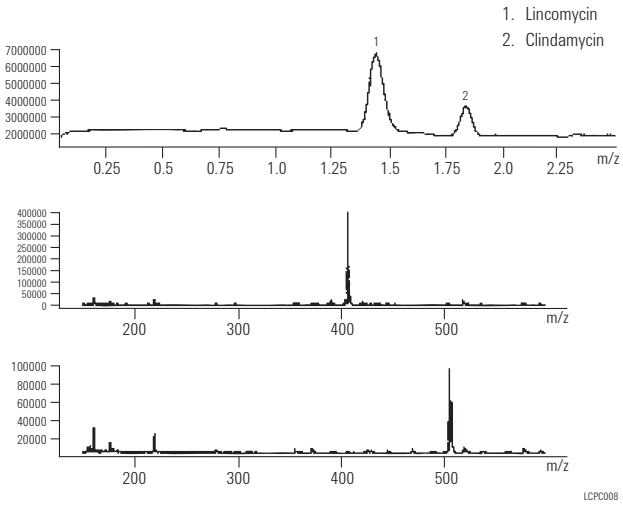
Temperature: Ambient

Detector: APCI, Positive ion

MS Conditions:

Peak width:	0.10 min
Scan:	150-600 Da, step 0.1
Fragmentor:	70
Gas Temp:	350 °C
Vaporizer:	350 °C
Drying gas:	12 L/min
Nebulizer pres:	50 psi
Vcap:	+3000 V
Corona:	4.0 µA

Sample: Antibiotics, 1 µL



Antifungal medications

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

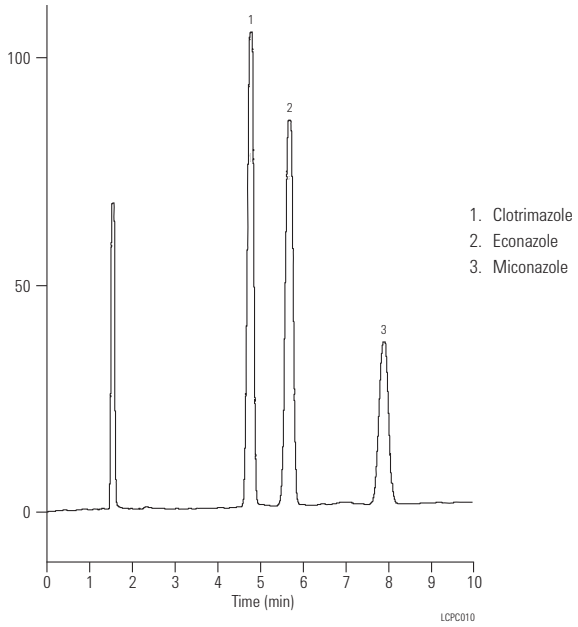
Mobile Phase: 35% 25 mM NaH_2PO_4, Dibasic (pH 6.5 with H_3PO_4): 65% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antifungals, 2 µL



1. Clotrimazole
2. Econazole
3. Miconazole

Antifungals

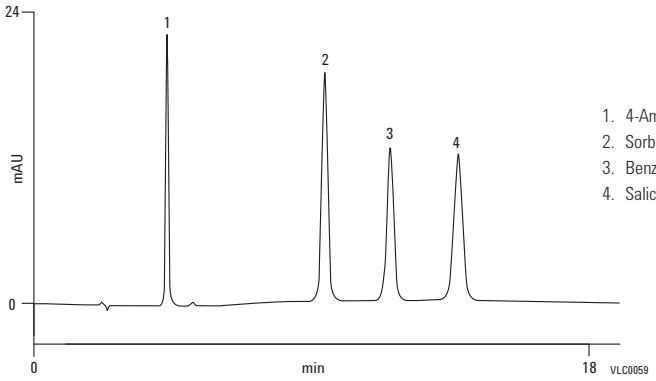
Column: **Pursuit XRs Diphenyl**
A6020150X046
4.6 x 150 mm, 5 µm

Mobile Phase: Water+0.1% HCOOH: MeCN+0.1% HCOOH, 80:20

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. 4-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid
4. Salicylic acid

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Analgesics: Non-steroidal anti-inflammatory drugs: Narrow bore separation

Column: **Eclipse XDB-C8**
993700-906
2.1 x 150 mm, 5 µm

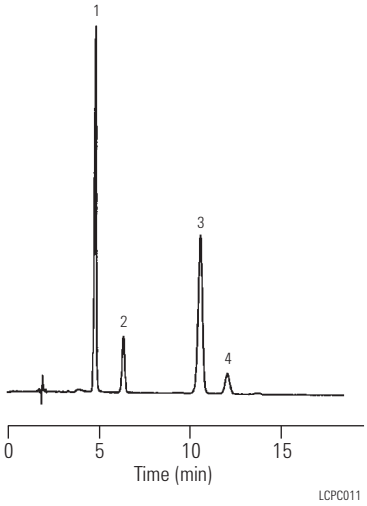
Mobile Phase: 50/50, 25 mM Sodium Phosphate (pH 7.0 with Phosphoric Acid), MeOH

Flow Rate: 0.2 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL, 10 ug/mL



NSAID	pK_a
1. Phenacetin	2.2
2. Tolmetin	3.5
3. Phenylbutazone	4.4
4. Fenoprofen	4.5

Separation of small molecule anorectics

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

Column B: **Traditional Alkyl C8 Phase**

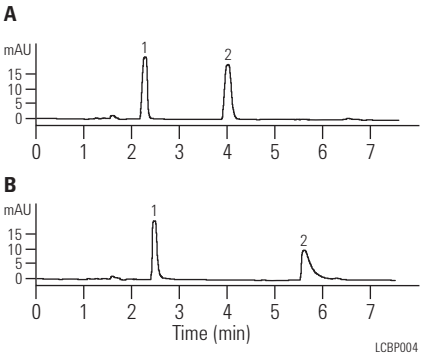
Mobile Phase: 25 mM K_2HPO_4, pH 7.2/MeOH: ACN (50:50), 45/55

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Anorectics "Fen-phen", 5 µL



1. Phentermine
2. Fenfluramine

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Aromatic acids/benzoic acids: Selectivity differences

Column A: **ZORBAX SB-C8**
880975-906
4.6 x 250 mm, 5 µm

Column B: **ZORBAX SB-Phenyl**
880975-912
4.6 x 250 mm, 5 µm

Column C: **ZORBAX SB-CN**
880975-905
4.6 x 250 mm, 5 µm

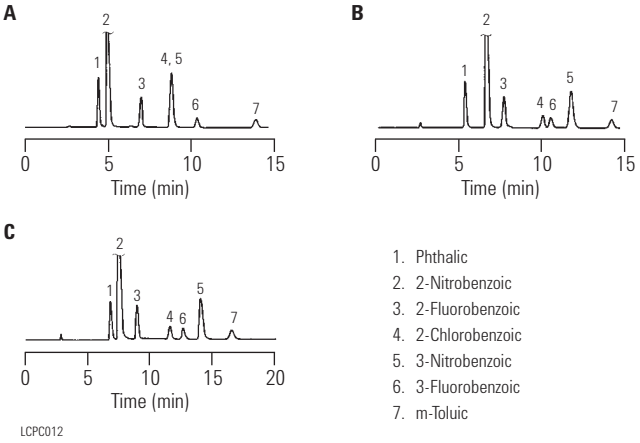
Mobile Phase: 30-45% methanol in 25 mM Na Phosphate, pH 2.5
A: 45% Methanol
B: 40% Methanol
C: 30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Benzoic acids



LCPC012

1. Phthalic
2. 2-Nitrobenzoic
3. 2-Fluorobenzoic
4. 2-Chlorobenzoic
5. 3-Nitrobenzoic
6. 3-Fluorobenzoic
7. m-Toluic

Catecholamines/biogenic amines: Rapid separation using ion-pair reagents

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

Mobile Phase: 0.14 M sodium phosphate,
20 mM EDTA,
0.75 mM octyl sulfonate,
9% methanol pH 3.5

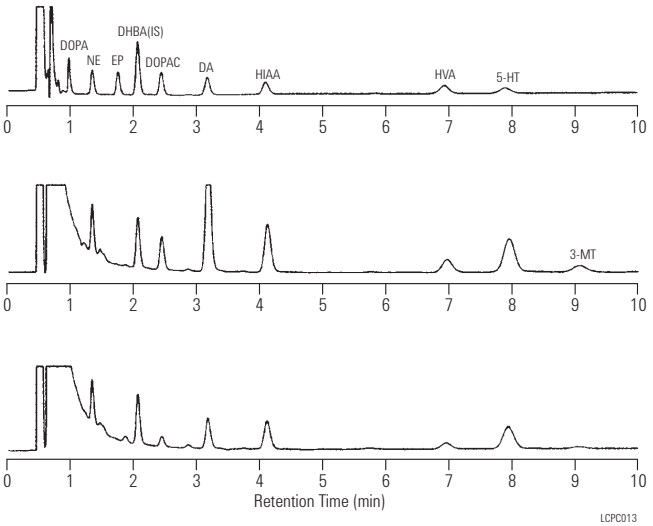
Flow Rate: 1.5 mL/min

Temperature: 26 °C

Detector: 0.75 V vs Ag/AgCl with electro-chemical detection

Sample: 10 µg/mL each standard; volume
20 µL (2 g tissue sample)
A: Standards (2pmol; DHBA 5pmol)
B: Mouse Sriatum
C: Mouse Neocortex

1. DOPA-Dihydroxyphenylalanine
2. DHBA-Dihydroxybenzyl amine
3. DOPAC-Dihydroxyphenyl acetic acid
4. NE-Norepinephrine
5. DA-Dopamine
6. HIAA-Hydroxyindoleaacetic acid
7. EP-Epinephrine
8. HVA-Homovanillic acid
9. 5-HT-Hydroxytryptamine
10. 3-MT-Methaxytyrosine



LCPC013

Chiral ethiazide (diuretic drug) separation

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

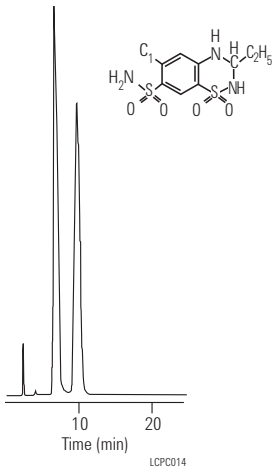
Mobile Phase: 20 mM KH_2PO_4 (pH 4.6)

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg Ethiazide



Chiral separation of fluoxetine enantioners (Prozac)

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

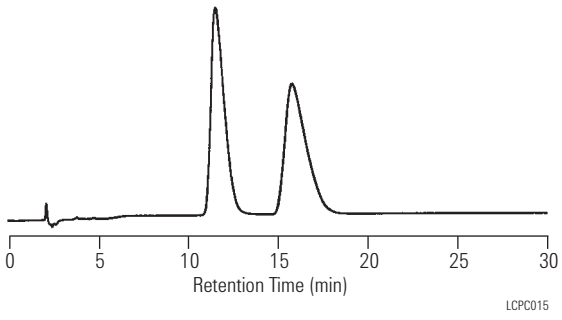
Mobile Phase: 25/75 (v/v) EtOH / 20 mM KH_2PO_4, pH 5.5 (adjusted with NaOH)

Flow Rate: 0.8 mL/min

Temperature: Ambient

Detector: UV, 225 nm

Sample: Mixture fluoxetine (Prozac) enantiomers



Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Goldenseal and related alkaloids on Rapid Resolution Eclipse XDB-C18

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

Mobile Phase: 68% 30 mM ammonium acetate,
14 mM TEA, pH ~4.85
32% Acetonitrile

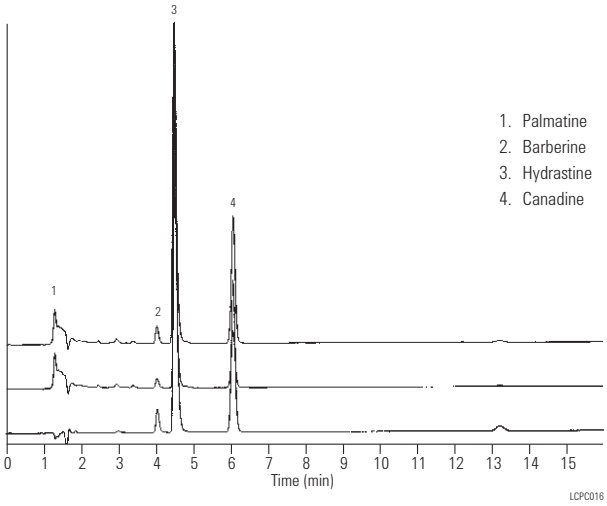
Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: 230 nm

Sample: Goldenseal and related alkaloids

Alkaloids, such as the active components in Goldenseal and other related plants, are quickly and accurately separated using isocratic conditions on an Eclipse XDB-C18 Rapid Resolution column.



1. Palmatine
2. Barberine
3. Hydrastine
4. Canadine

Components of green tea separated on Rapid Resolution StableBond SB-C8

Column: **ZORBAX SB-C8**
863953-906
4.6 x 150 mm, 3.5 µm

Mobile Phase: 75% 0.1% TFA : 25% MeOH

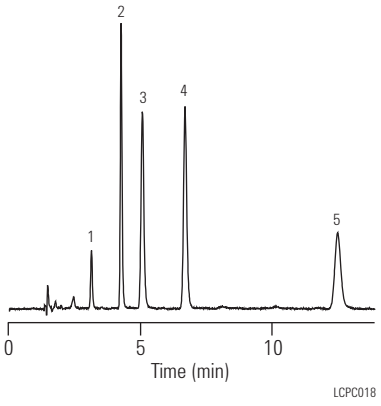
Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: 280 nm

Sample: Green tea

Nutraceuticals, such as the components of green tea, are quickly separated on a StableBond SB-C8 Rapid Resolution column.



1. Epigallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

Chiral separation of hexobarbitol

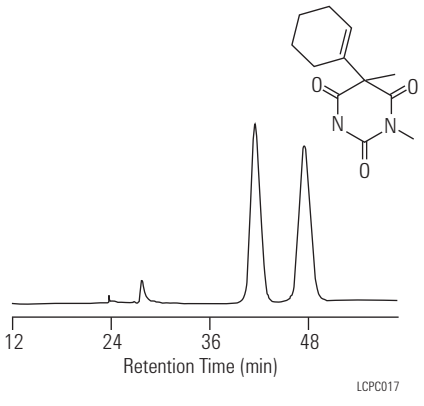
Column: **Chiradex**
79925CB-584
4.0 x 250 mm, 5 µm

Mobile Phase: Methanol/water, 20:80

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

Sample: Hexobarbitol



Chiral separation of S- and R-Norfluoxetine

Column: **Ultron ES-OVM Chiral**
724111653
4.6 x 250 mm, 10 µm

Mobile Phase: 6/94 (v/v) MeOH / 20 mM KH_2PO_4

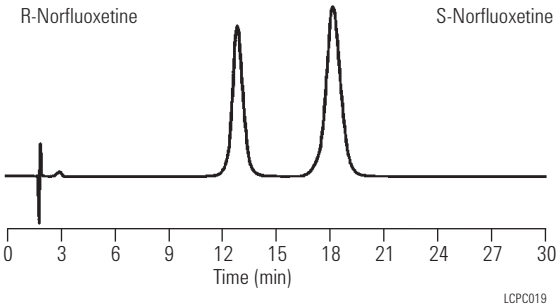
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 225 nm

Sample: 50 µg/mL of 2:3 mixture R- : S-Norfluoxetine

Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.



Chiral separation of salbutamol

Column: **Ultron ES-Pepsin**
822111631A
4.6 x 150 mm, 5 µm

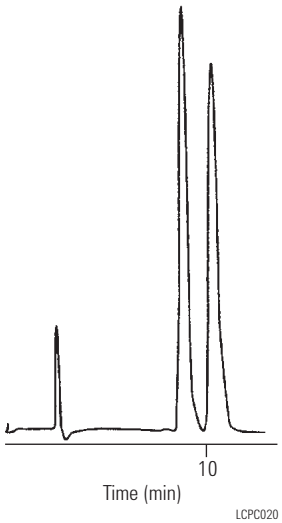
Mobile Phase: 20 mM phosphate buffer, pH 6.0

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg salbutamol mixture



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Chiral separation of tolperison enantiomers

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

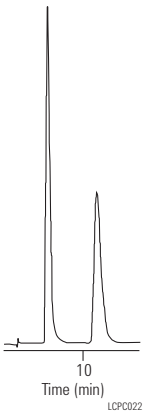
Mobile Phase: 20 mM KH_2PO_4 (pH 5.5), C_2H_5OH (100/4 v/v)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm, 0.04 AUFS

Sample: Tolperison, 5 µL



Chiral separation of atenolol

Column: **Ultron ES-Pepsin**
822111631A
4.6 x 150 mm, 5 µm

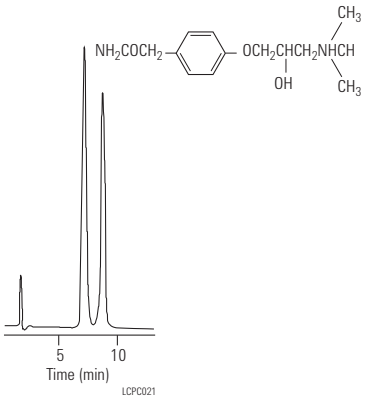
Mobile Phase: 20 mM phosphate buffer, pH 6.0/Ethanol (99/1)

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm, 0.04 AUFS

Sample: 1.5 µL, 0.25 mg/mL, atenolol racemic mixture



Cocaine and metabolites

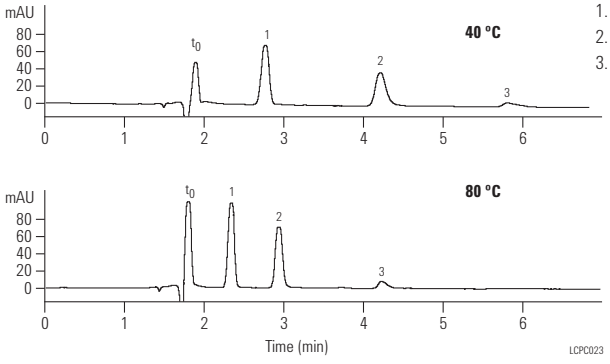
Column: **ZORBAX Rx-SIL**
883975-901
4.6 x 150 mm, 5 µm

Mobile Phase: MeOH: NH_4 Acetate, 25 mM, pH 6 (70:30)

Flow Rate: 1.0 mL/min

Temperature: 40 and 80 °C

Detector: UV, 210 nm



1. Benzoylecgonine
2. Cocaine
3. Ecgonine methylester

Aspirin and cough remedy

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

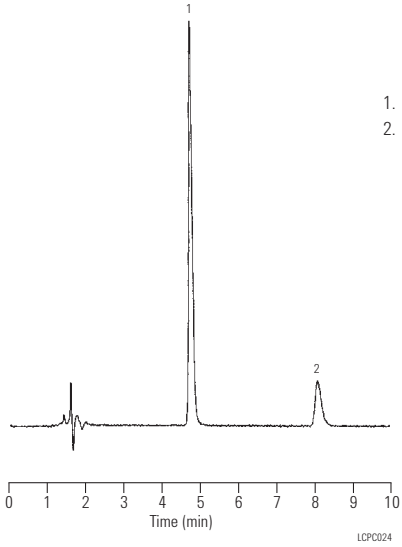
Mobile Phase: (75:25) 25 mM Na_2HPO_4 (pH 3.0): ACN

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: UV, 254 nm

Sample: 5 µL, 10 µg/mL



1. Acetylsalicylic acid
2. Dextromethorphan

Cough formula mixture: Fast and efficient separation

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

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

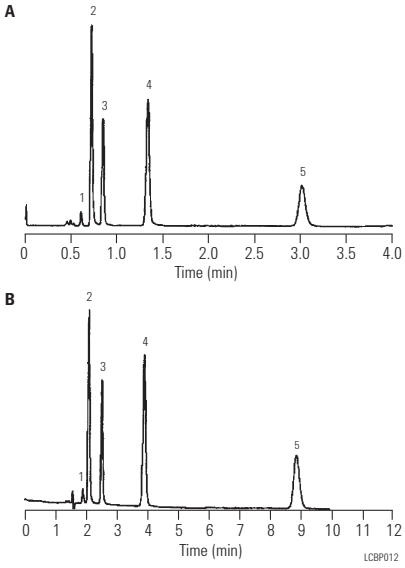
Mobile Phase: 20/80, Acetonitrile/150 mM Na Citrate, pH 2.6

Flow Rate: 1.5 mL/min, 1.0 mL/min

Temperature: 35 °C

Detector: UV, 270 nm

Sample: 2 µL, cough formula



1. Maleic acid
2. Pseudo-ephedrine
3. Acetaminophen
4. Chlorpheniramine
5. Dextromethorphan

Guaifenesin: USP analysis of guaifenesin

Mobile Phase: 40% Methanol:60% Water:1.5% Glacial Acetic Acid

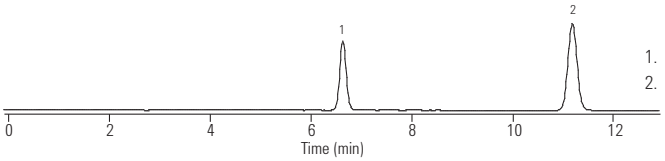
Flow Rate: 1.0 mL/min

Temperature: 25 °C

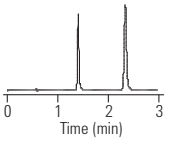
Sample: Guaifenesin
A: 8 µL
B: 2 mL

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

Peak	TR	N	Rs
1	6.63	12,737	0
2	11.19	18,552	15.8



1. Guaifenesin: 0.04 mg/mL
2. Benzoic Acid: 0.10 mg/mL



Column: **Eclipse XDB-C18**
922975-902
4.6 x 50 mm, 1.8 µm

Peak	TR	N	Rs
1	1.4	11,421	0
2	2.33	12,909	12.3

Minimum Resolution Required = 3.0

Metronidazole: Updating USP methods

Column A: **ZORBAX C8**
883952-706
4.6 x 150 mm, 5 µm

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

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

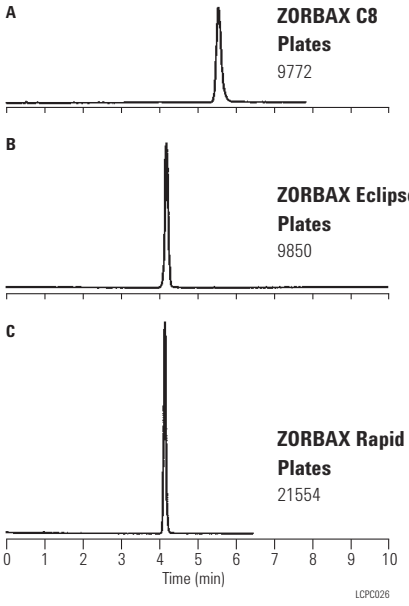
Mobile Phase: 80/20, Water/Methanol

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Metronidazole



A **ZORBAX C8**

Plates	USP TF	Particle Size
9772	1.30	5 µm

B **ZORBAX Eclipse XDB-C8**

Plates	USP TF	Particle Size
9850	0.98	5 µm

C **ZORBAX Rapid Resolution Eclipse XDB-C8**

Plates	USP TF	Particle Size
21554	1.13	3.5 µm

Morphine and metabolites: Extracted blood plasma sample separation

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

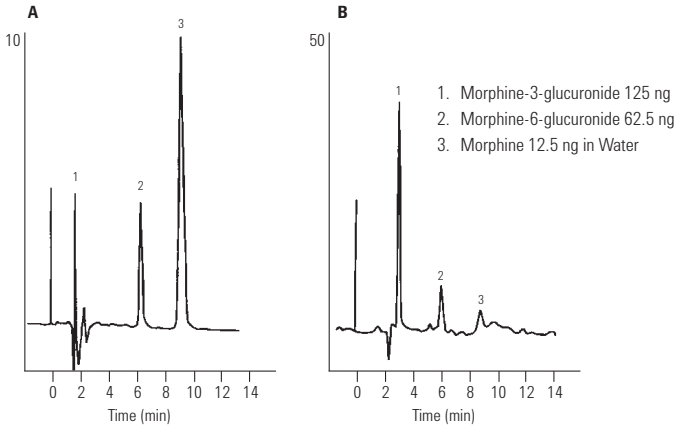
Mobile Phase: 97/3 70 mM KH_2PO_4 + 1 mM EDTA/ACN, pH 4.5

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: A: Electrochemical, 720 mV
B: Fluorescence, Ex = 285 nm, Em = 352 nm

Sample: 50 µL
Morphine-3-glucuronide 125 ng
Morphine-6-glucuronide 62.5 ng
Morphine 12.5 ng in Water



Courtesy of J. Visser, Center for Pharmacy, Univ. Groningen, The Netherlands.

Opiates (drugs of abuse) by LC/MS

Column: **ZORBAX SB-AQ**
830990-914
2.1 x 150 mm, 3.5 µm

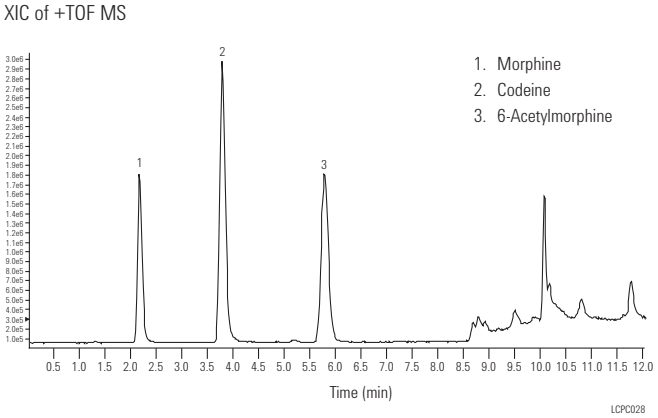
Mobile Phase: A: Acetonitrile with 0.1% formic acid
B: Water with 0.1% formic acid

Flow Rate: 0.25 mL/min

Gradient: 0 min 10% B
5 min 35% B
5.1 min 100% B

MS Conditions: Time of Flight (TOF)
Standard with calibrant delivery system providing constant low flow of ~ 2 µM purine and HP-921 calibrant to dual ESI for continuous auto-calibration

Sample: Opiates



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NEW!

Comparing HILIC and RPLC of morphine using Agilent ZORBAX RRHD columns with UHPLC/MS

Column: **Agilent ZORBAX Eclipse Plus C18**
2.1 x 100mm, 5 µm
(Custom column)

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 10 mM NH_4HCO_2, pH 3.2
B: CH_3CN/100 mM NH_4HCO_2, pH 3.2 (9:1)
Column A: 10% B isocratic
Column B: 70% B isocratic

Flow Rate: Column A: 0.4 mL/min
Column B: 1 mL/min

Pressure: Column A: 90 bar
Column B: 810 bar

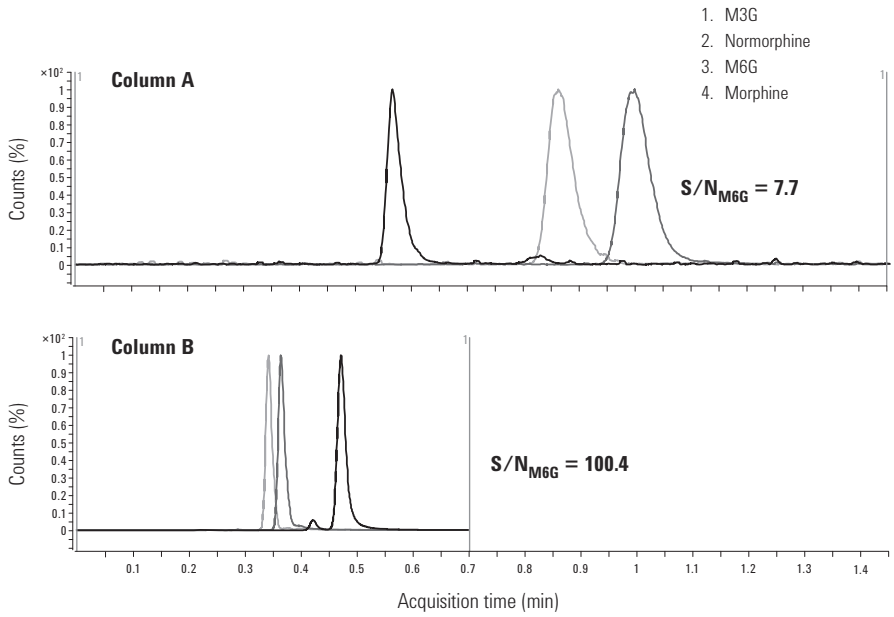
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC with an Agilent 6410A Triple Quadrupole Mass Spectrometer

MS Conditions:
MS Source: Positive ESI, capillary 4000 V, drying gas temperature, flow rate and nebulizer pressure vary with mobile phase flow rate
MS Acquisition: Selected ion mode (SIM), delta EMV 200 V, MS dwell time caries with mobile phase flow rate
Software: Agilent MassHunter versions B.03.01, B.02.00 AND B.03.01 were used for data acquisition, qualitative, and quantitative analyses, respectively

Sample: 2 µL injection of 1 µg/mL each of morphine, normorphine, morphine-3-ß-D-glucuronide: HILIC sample was prepared in CH_3CN; RPLC sample was prepared in H_2O

HILIC mode with UHPLC columns cuts analysis time in half, while improving sensitivity by more than a factor of 10, compared to traditional LC columns in RPLC mode with MS detection.



Neutraceuticals: Hypericin separation in St. John's Wort

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

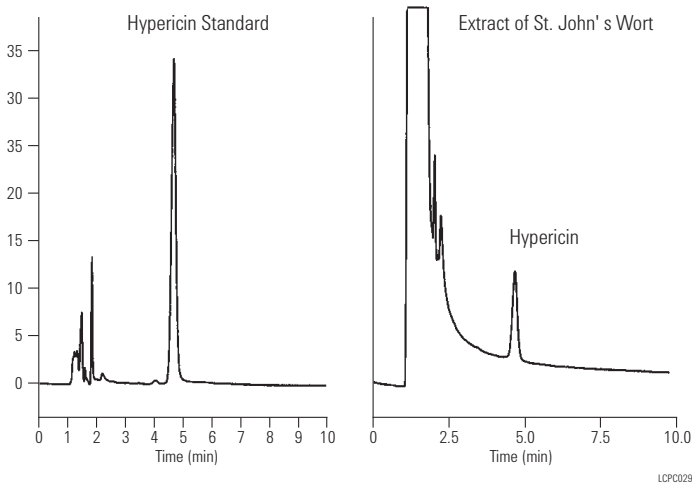
Mobile Phase: 23% 25 mM Na_2HPO_4 ,
Dibasic (pH 7.0 with H_3PO_4): 77% MeOH

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Neutraceuticals



Pharmaceuticals: Rapid, high sensitivity LC and LC/MS of 11 drugs

Column: **Eclipse XDB-C18**
925700-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: 10 mM NH_4 Formate (pH = 3.6)
B: ACN with 10 mM NH_4 Formate

Flow Rate: 0.6 mL/min

Gradient: 5% B to 70% B in 7.5 min, to 95% B in 8.5 min

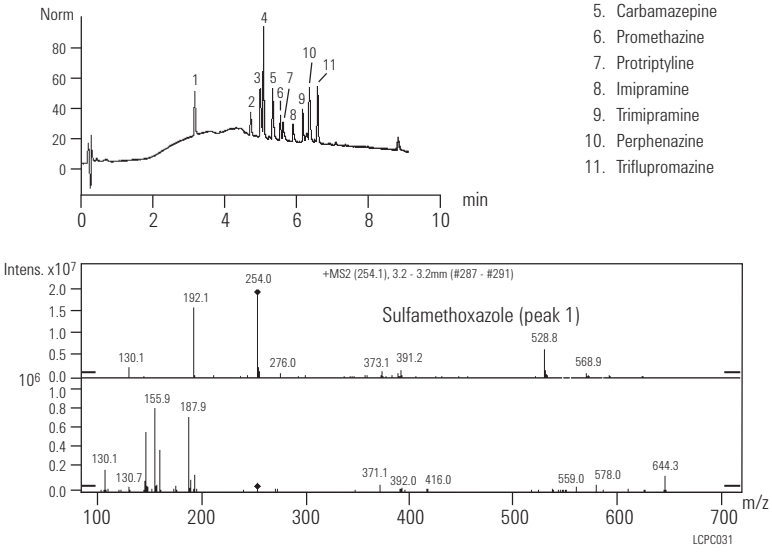
Temperature: 65 °C

Detector: UV, 230 nm and MSD Trap SL

MS Conditions:

Pos. Dry Gas:	345 °C
Neb.:	45 psi
HV Cap:	3500 V
Range:	100-700
Average:	5 Spectra
ICC:	30000
Charge Con:	On
Smart Par. Settings:	Tar Mas: 250 m/z
Comp. Stab.:	100%
Trap Drive:	100%
Frag. Options:	Smart Frag: On
Frag. Width:	10 m/z

1. Sulfamethoxazole
2. Tripelennamine
3. Prednisolone
4. Diphenhydramine
5. Carbamazepine
6. Promethazine
7. Protriptyline
8. Imipramine
9. Trimipramine
10. Perphenazine
11. Triflupromazine



Hormones/steroids

Column: **ZORBAX RRHT SB-C18**
823975-902
4.6 x 30 mm, 1.8 µm

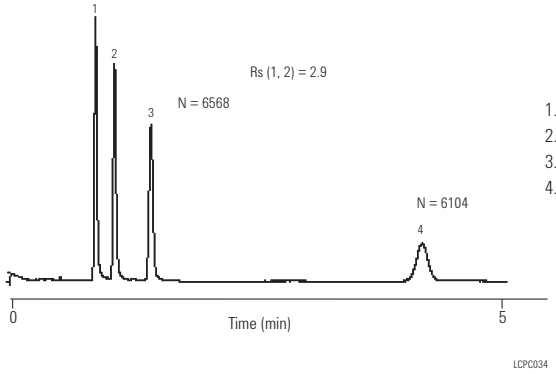
Mobile Phase: 50% 20 mM NaH_2PO_4, pH 2.8: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: RT

Detector: UV, 230 nm

Sample: Hormones/steroids



1. Estradiol
2. Ethinylestradiol
3. Dienestrol
4. Norethindrone

Steroids: Separation

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

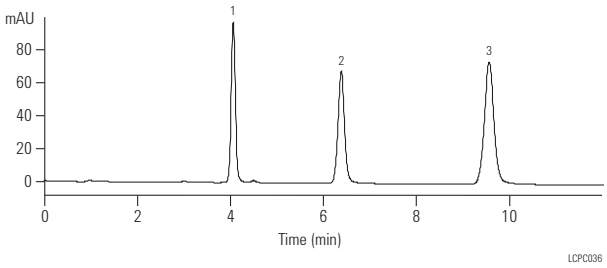
Mobile Phase: 40:60 ACN:Water

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 205 nm

Sample: 1. Norethindrone 0.514 mg/mL
2. Progesterone 0.407 mg/mL
3. Mestranol 0.057 mg/mL



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Steroids

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

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

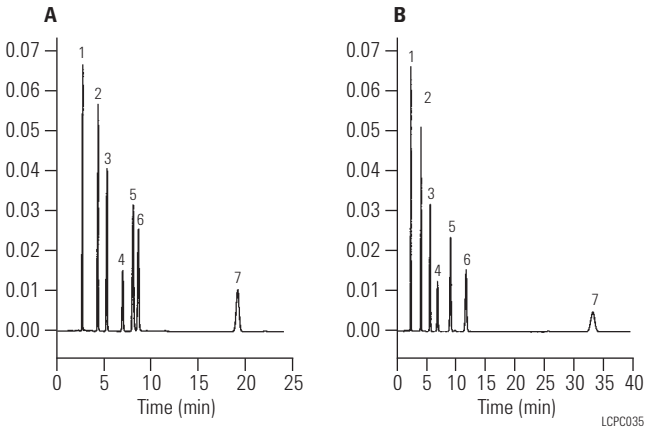
Mobile Phase: H_2O:ACN, 60:40

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample:
1. Prednisolone
2. Corticosterone
3. 11 -hydroxyprogesterone
4. Cortisone acetate
5. Deoxycorticosterone
6. 17 hydroxyprogesterone
7. Progesterone



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Triamcinolone – USP analysis of triamcinolone

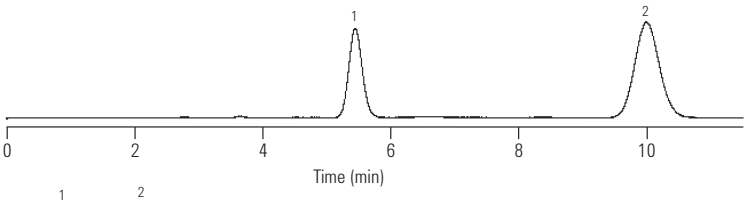
Column: **Eclipse XDB-C18**
923975-902
4.6 x 30 mm, 1.8 µm

Mobile Phase: 47% Methanol:53% Water

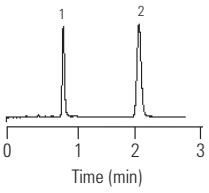
Flow Rate: 1.5 mL/min

Temperature: 25 °C

Sample: Triamcinolone, 1 µL



Peak	TR	N	Rs
1	5.45	3199	0
2	9.99	3212	8.1



1. Triamcinolone: 0.2 mg/mL
2. Hydrocortisone: 0.3 mg/mL

Minimum Resolution Required = 3.0

Peak	TR	N	Rs
1	0.89	3256	0
2	2.07	4851	11.8

LCPC038

Separation of highly basic antidepressants above their pKa in free base form (pKa 9.5-9.7)

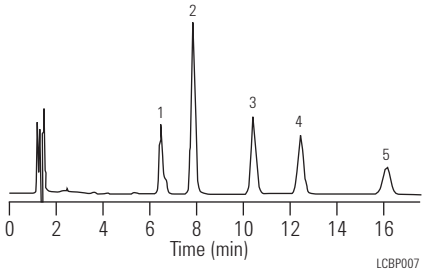
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

Mobile Phase: 75% Methanol / 25% 50 mM Pyrrolidine Buffer, pH 11.5

Flow Rate: 0.5 mL/min

Temperature: 40 °C

Detector: UV, 215 nm



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

LCBP007

Basic drugs can often be separated in their charged form at low pH with StableBond or at mid-range pH with Eclipse XDB or Bonus -RP columns. With Extend-C18, you can separate at high pH to improve solubility, improve retention, or obtain different selectivity.

Antidepressants, tricyclic: Comparative separation

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

Column B: **Brand A Polar-linked C8**

Column C: **Brand B Polar-linked C18**

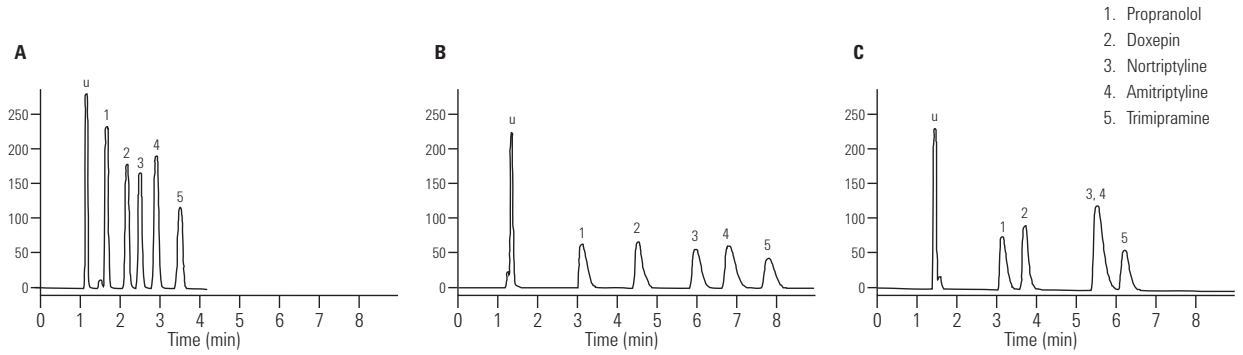
Mobile Phase: ACN: 20 mM Na Citrate, pH 6 (60:40)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Tricyclic antidepressants (u= uracil)



LCBP011

Tricyclic antidepressants

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

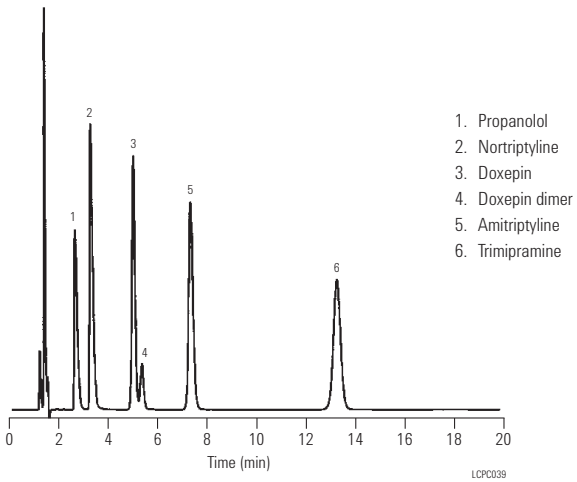
Mobile Phase: 38/62 THF/25 mM Potassium Phosphate, pH7

Flow Rate: 1.0 mL/min

Temperature: 23 °C

Detector: UV, 254 nm

Sample: 10 µL, Antidepressant mix, 10 µg/mL



LCPC039

Tricyclic antidepressants and metabolites: Effect of pore size

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

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

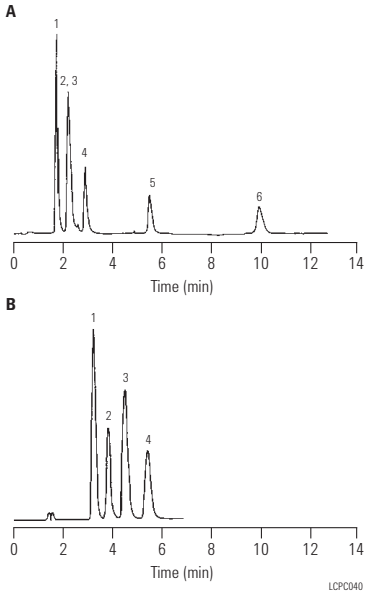
Mobile Phase: 40/60, 25 mM Phosphate Buffer, 10 mM Triethylamine, pH 6.2/ACN

Flow Rate: 1.2 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 10 µL, Antidepressant mix, 10 µg/mL



1. trans- 10-OH - Nortriptyline
2. trans- 10-OH - Amitriptyline
3. cis- 10-OH - Nortriptyline
4. cis- 10-OH - Amitriptyline
5. Nortriptyline
6. Amitriptyline

Ulcer treatment drugs at intermediate pH

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

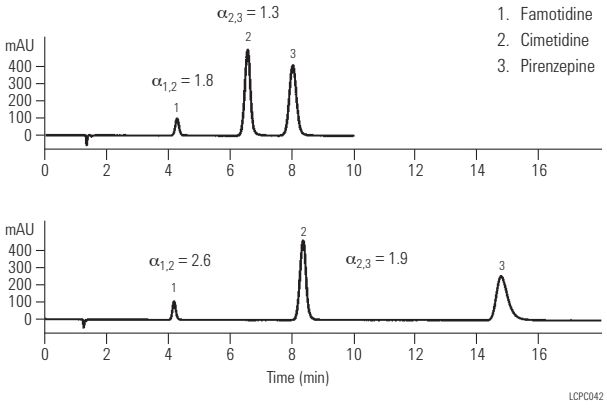
Mobile Phase: Na citrate, 20 mM, pH 6.1: MeOH, (80:20)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Ulcer treatment drugs



1. Famotidine
2. Cimetidine
3. Pirenzepine

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Urine, LSD analysis by LC/MS

Column: **Eclipse XDB-C8**
960967-906
2.1 x 50 mm, 5 µm

Mobile Phase: 15 : 85, ACN : 10 mM Ammonium Formate, pH 3.7

Flow Rate: 0.3 mL/min

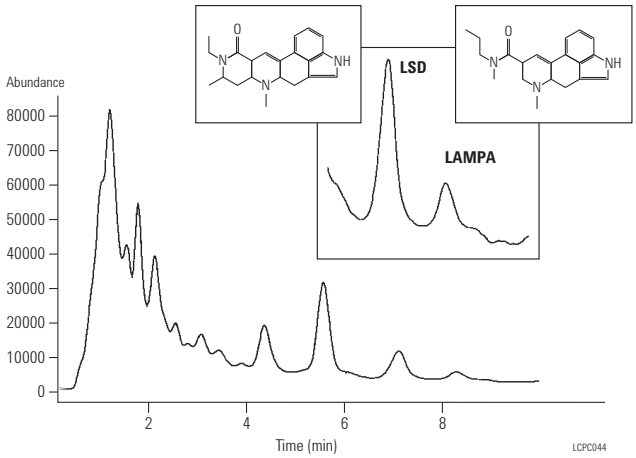
Temperature: 30 °C

Detector: MS

MS Conditions: SIM mode, Ions: 324.2, 223.1, 208.1
Fragmentor (dynamically ramped) 100V at 324.2, 148V at 223.1, 170V at 208.1

Sample: LSD

Hughes, J.M., C.A. Miller and S.M. Fischer, "Development of a Method for the Forensic Analysis of LSD in Urine", presented at the ASMS, Palm Springs, June 1997.



USP method:
Glyburide and internal standard, progesterone

Column: **Eclipse XDB-C8**
990967-906
4.6 x 250 mm, 5 µm

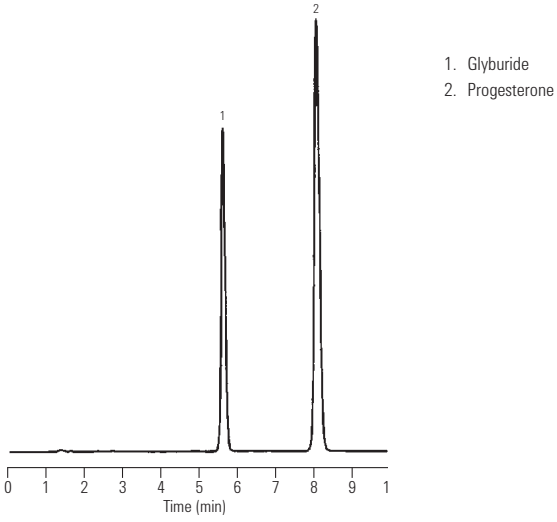
Mobile Phase: 45/55, 50 mM Ammonium Phosphate/ACN, Final pH 5.35

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 5 µL, 10 ug/mL each of standard



1. Glyburide
2. Progesterone

Dexamethasone, USP method: Rapid analysis

Column A: **ZORBAX SB-C8**
880975-906
4.6 x 250 mm, 5 µm

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

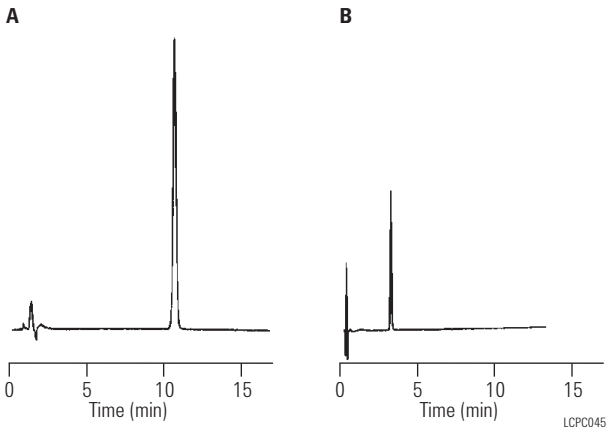
Mobile Phase: A = Water, B = ACN; Isocratic 30% B

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Dexamethasone
10 µL and 5 µL, 10 ug/mL



USP analysis of tetracyclines

Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

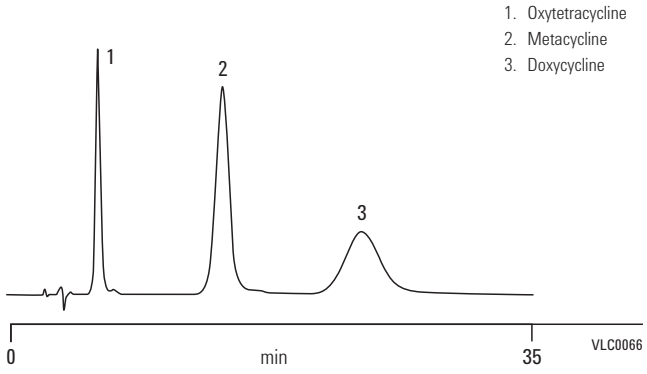
Sample: 20 mg tetracycline in 25 mL 0.01M HCl

Mobile Phase: 60 g 2-Methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K_2HPO_4 at pH 8 + 50 mL 10 g/L tetrabutylammonium hydrogen sulphate at pH 8 + 10 mL 40 g/L sodium edetate at pH 8, made up to 1000 mL with water (adjust pH with dilute NaOH)

Flow Rate: 1.0 mL/min

Temperature: 60 °C

Detector: UV, 254 nm



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Warfarin: USP chromatographic purity method using Eclipse XDB-CN

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

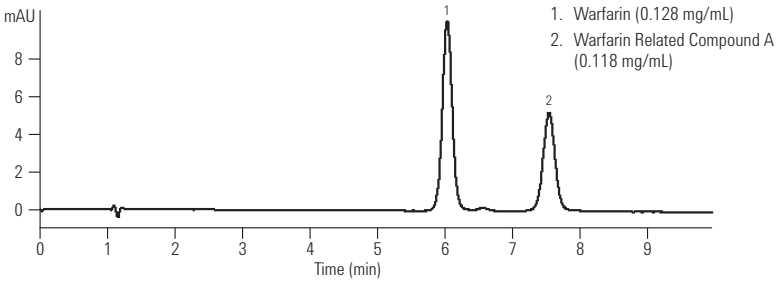
Mobile Phase: 32:68:1 Acetonitrile:Water:Glacial Acetic Acid

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Detector: UV, 260 nm

Sample: Warfarin, 2 µL



Ten cardiac drugs on Rapid Resolution HT SB-C18

Column: **SB-C18**
829975-902
4.6 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

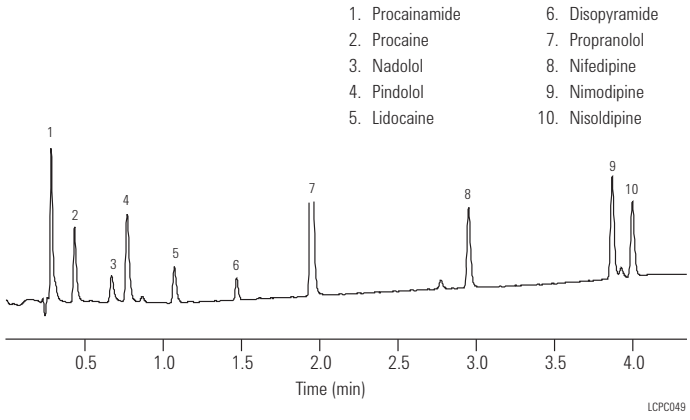
Flow Rate: 2 mL/min

Gradient: 0.0 min 12.5% B
10.5 min 60% B
12.0 min 60% B

Temperature: 70 °C

Detector: UV, 230 nm

Sample: Cardiac drugs



Sulfonamides – Fast analysis with RRHT columns

Column: **SB-C18**
824700-902
2.1 x 30 mm, 1.8 µm

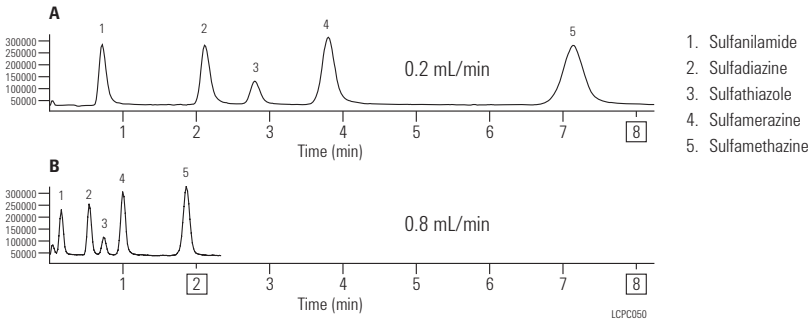
Mobile Phase: A: 90% 0.1% formic acid
B: 10% 0.1% formic acid in MeOH

Flow Rate: A: 0.2 mL/min
B: 0.8 mL/min

Temperature: 35 °C

Detector: TIC, Single Quad

Sample: Sulfonamides



Sulfa drugs

Column: **Pursuit XRs Ultra C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

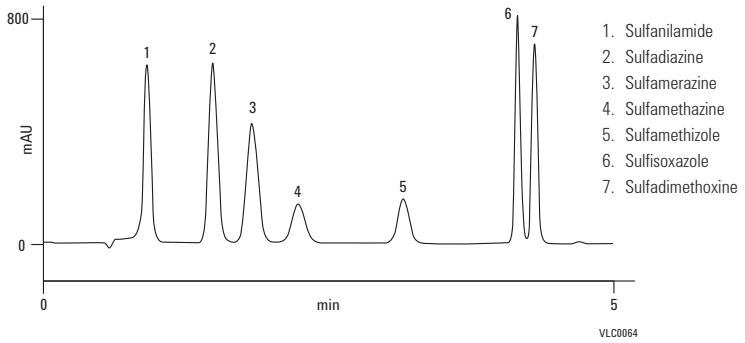
Mobile Phase: A: Water+0.1% TFA
B: MeCN+0.1% TFA

Gradient: 10% B for 10 min,
ramp to 45% B in 1 min and hold for 1 min,
return to 10% B in 1 min and hold for 1 min

Flow Rate: 0.65 mL/min

Temperature: Ambient

Detector: UV, 254 nm



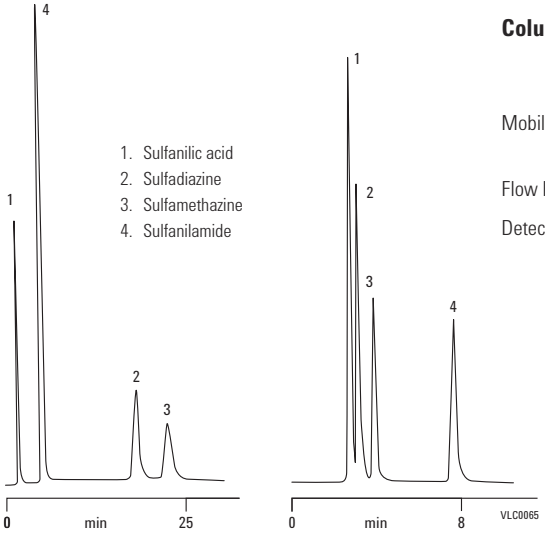
Sulfa drugs

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: Potassium sulfate:
ACN 7:1, pH 2.2

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm



Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

Mobile Phase: Disodium tetraborate: ACN 6:1,
pH 9.3

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm

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Fast analysis of Pindolol

Column A: **ZORBAX SB-CN**
863953-905
4.6 x 150 mm, 3.5 µm

Column B: **ZORBAX SB-CN**
827975-905
4.6 x 50 mm, 1.8 µm

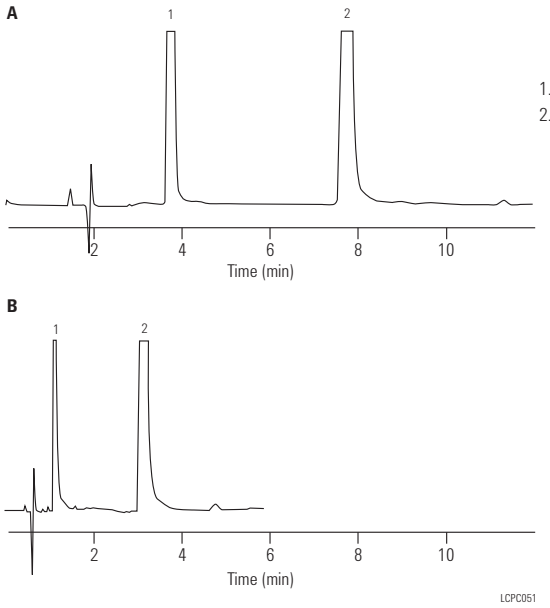
Mobile Phase: A: 70% 50 mM Na Acetate
B: 30% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 219 nm

Sample: Pindolol, 2 µL



1. Indole
2. Pindolol

Lamotrigine

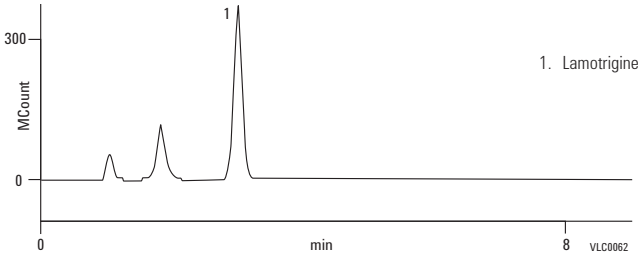
Column: **Pursuit XRs Ultra C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

Mobile Phase: ACN:water, 25:90 for 1 min

Flow Rate: 0.2 mL/min

Injection Volume: 5 µL, 50% MeOH

Detector: MS



1. Lamotrigine

Barbiturates

Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

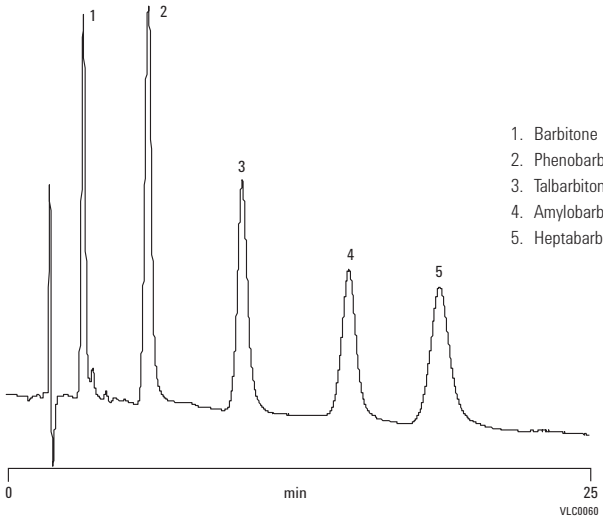
Mobile Phase: Water

Flow Rate: 1.0 mL/min

Temperature: 200 °C

Detector: UV, 220 nm

Courtesy: Smith, RM, Burgess, RJ, Cheinthavorn, O and Stuttard, JR (1999) Superheated water: a new look at chromatographic eleunts for reversed-phase liquid chromatography. LCGC Europe, January 1999, 30-36. Used with permission.



1. Barbitone
2. Phenobarbitone
3. Talbarbitone
4. Amylobarbitone
5. Heptabarbitone

Analysis of ciprofloxacin and ciprofloxacin metabolites

Column: **PLRP-S 100Å**
PL1111-3500
4.6 x 150 mm, 5 µm

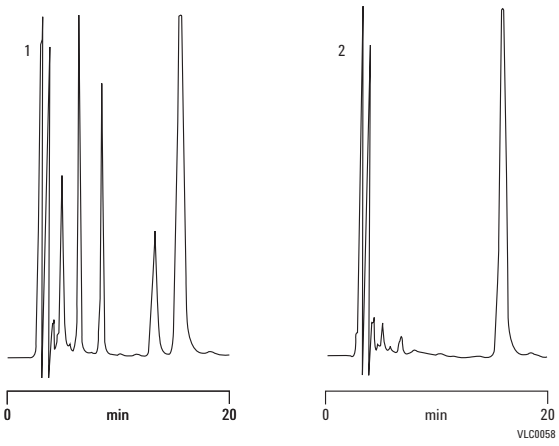
Mobile Phase: 74% 20 mM TCA:22% ACN:4% MeOH adjusted to pH 3

Flow Rate: 1.0 mL/min

Detector: UV, 277 nm

Krol GJ, Noe, AJ and Beerman, D (1986) Liquid chromatographic analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids. Journal of Liquid Chromatography, 9(13), 2897-2919. Reprinted with permission of the publisher (Taylor & Francis Group, www.informaworld.com).

1. Blank urine sample containing known concentrations of internal standard, ciprofloxacin and its metabolites
2. Blank urine sample containing only internal standard



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