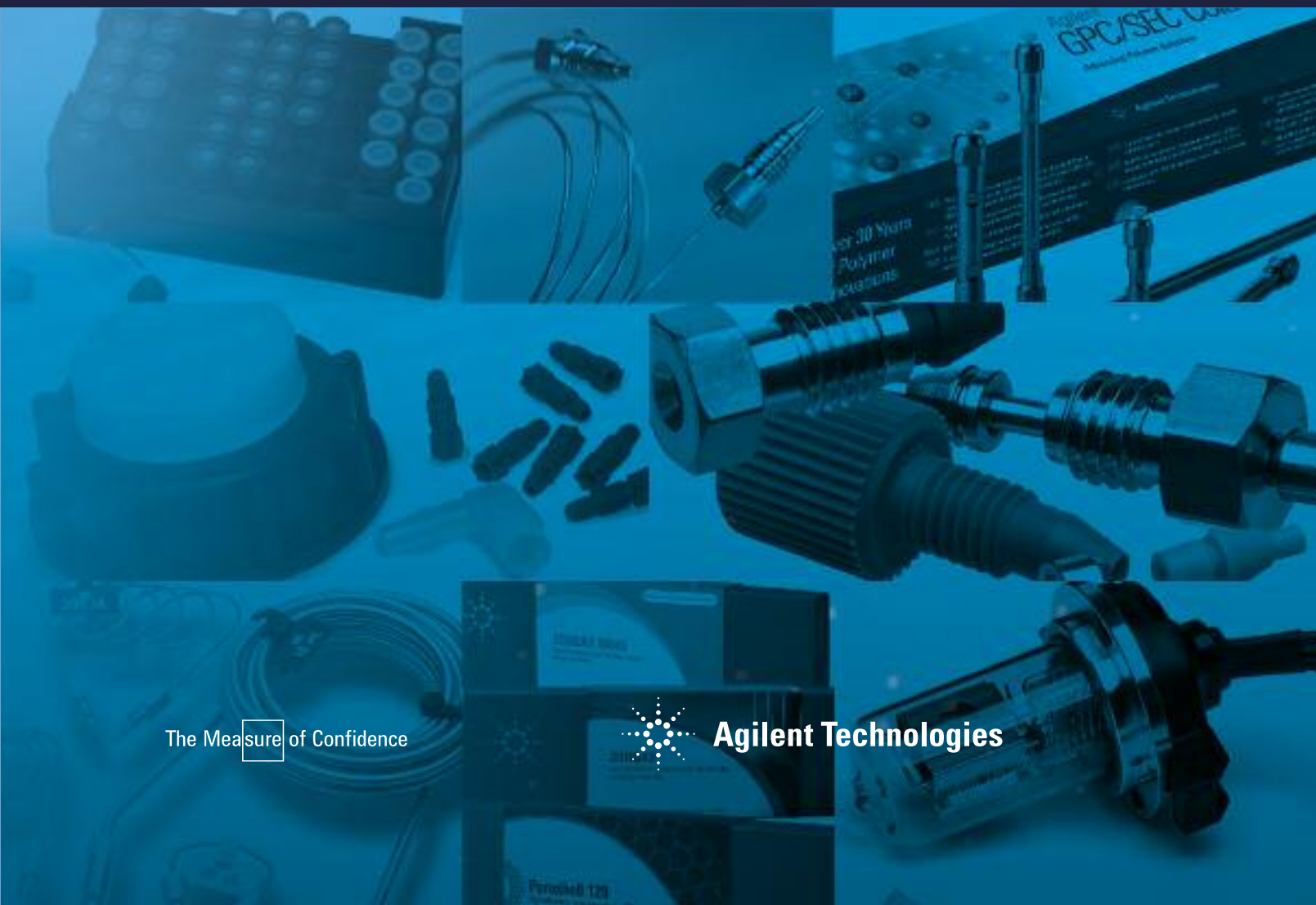
LC AND LC/MS

Your Essential Resource for Columns & Supplies



The Measure of Confidence

Agilent Technologies

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.

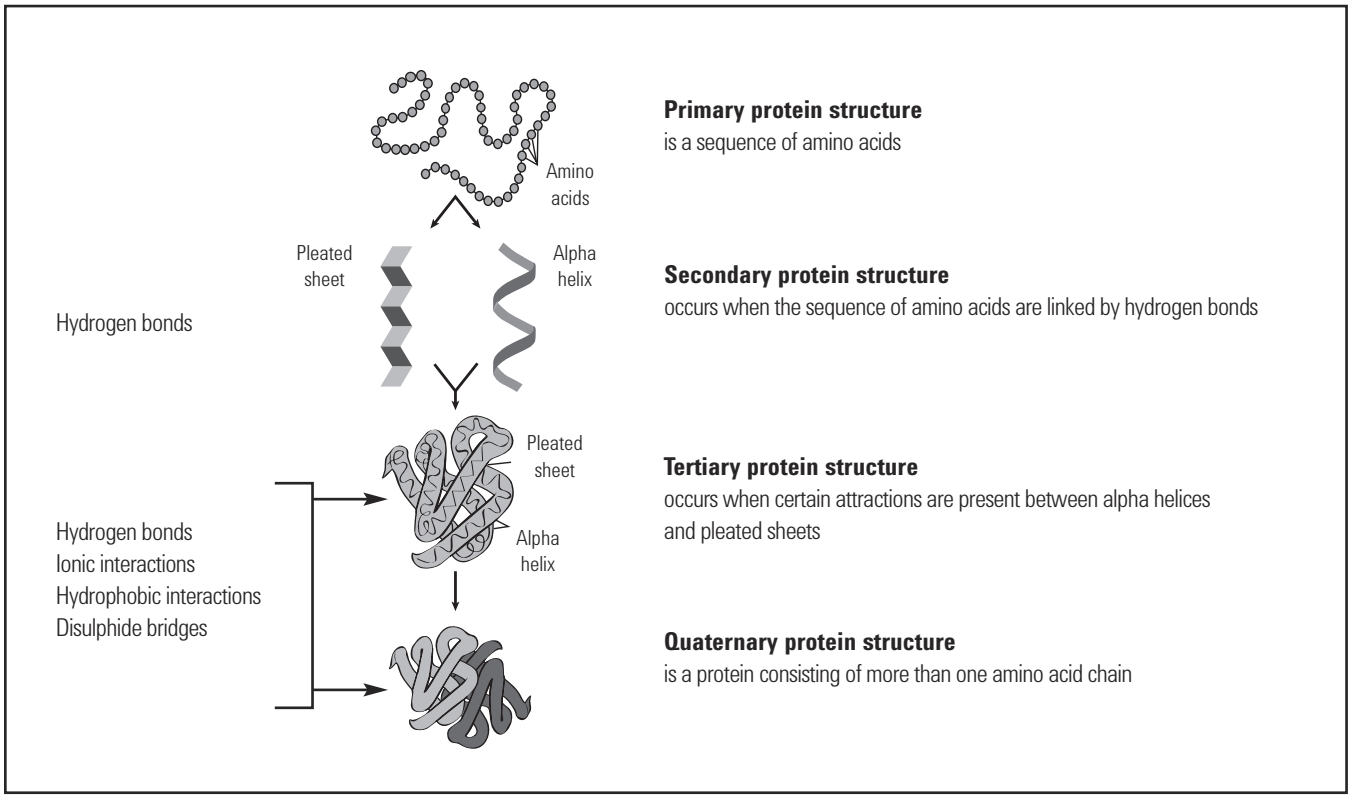


Figure 1. Schematic showing the various levels of protein structure.

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**

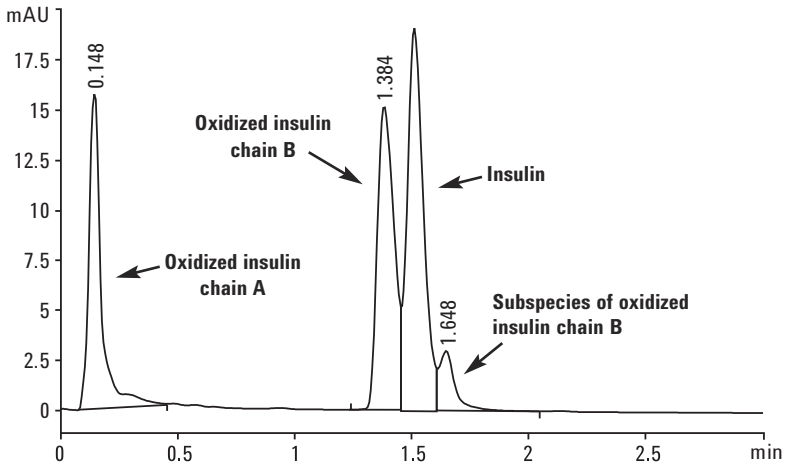
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

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

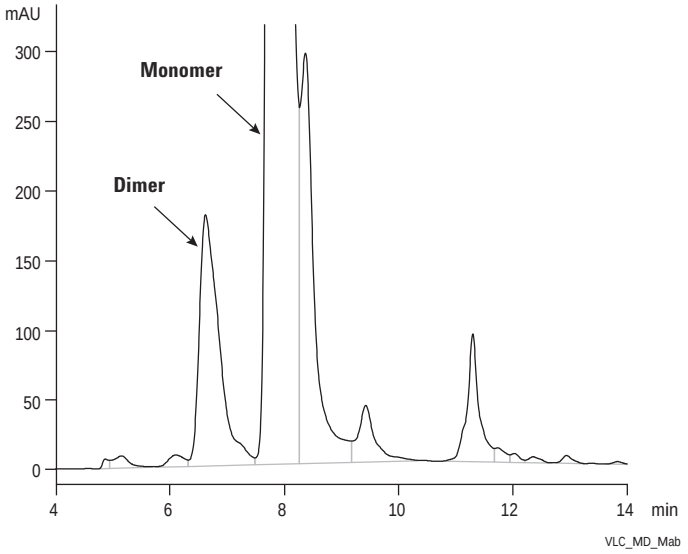
Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)



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.

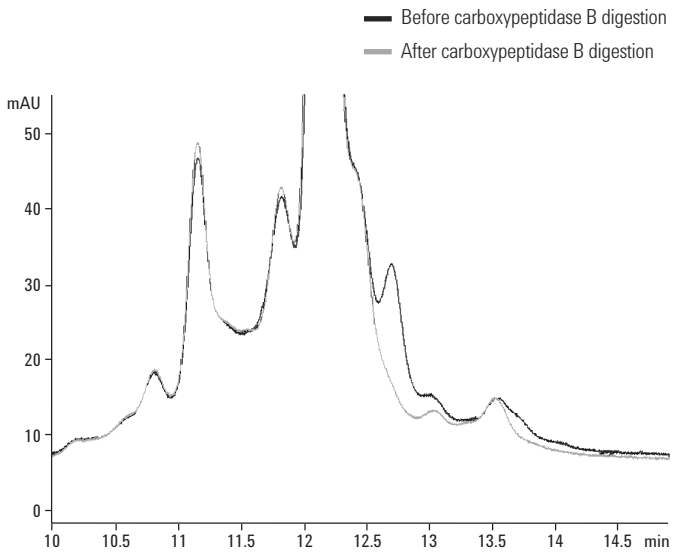
Intact MAb monomer and dimer separation

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

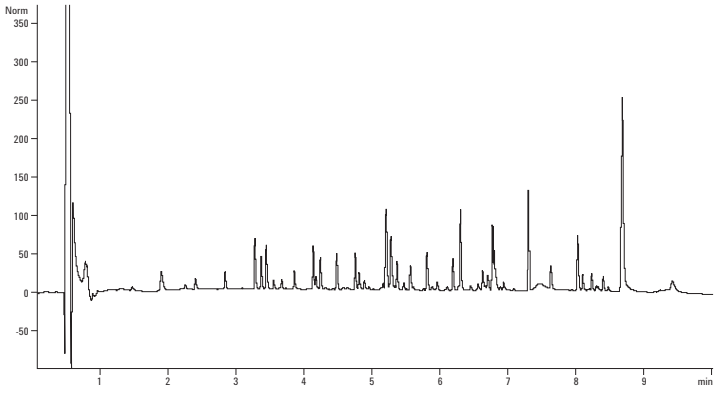
Flow Rate: 0.5 mL/min

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.

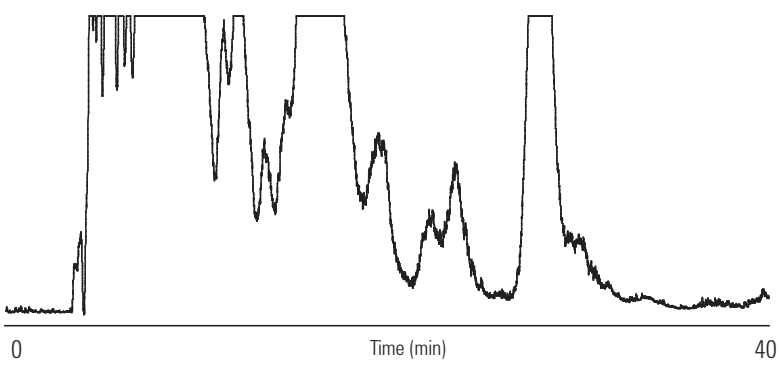
Preparative scale purification of Leuprolide by concentration overload

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

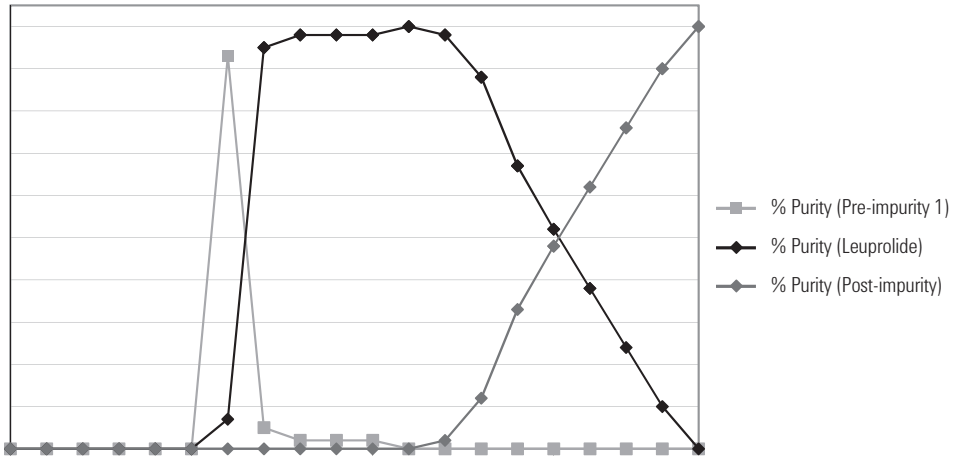
Bulk Media: **Load & Lock 4001 Column PCG93LL500X25**

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

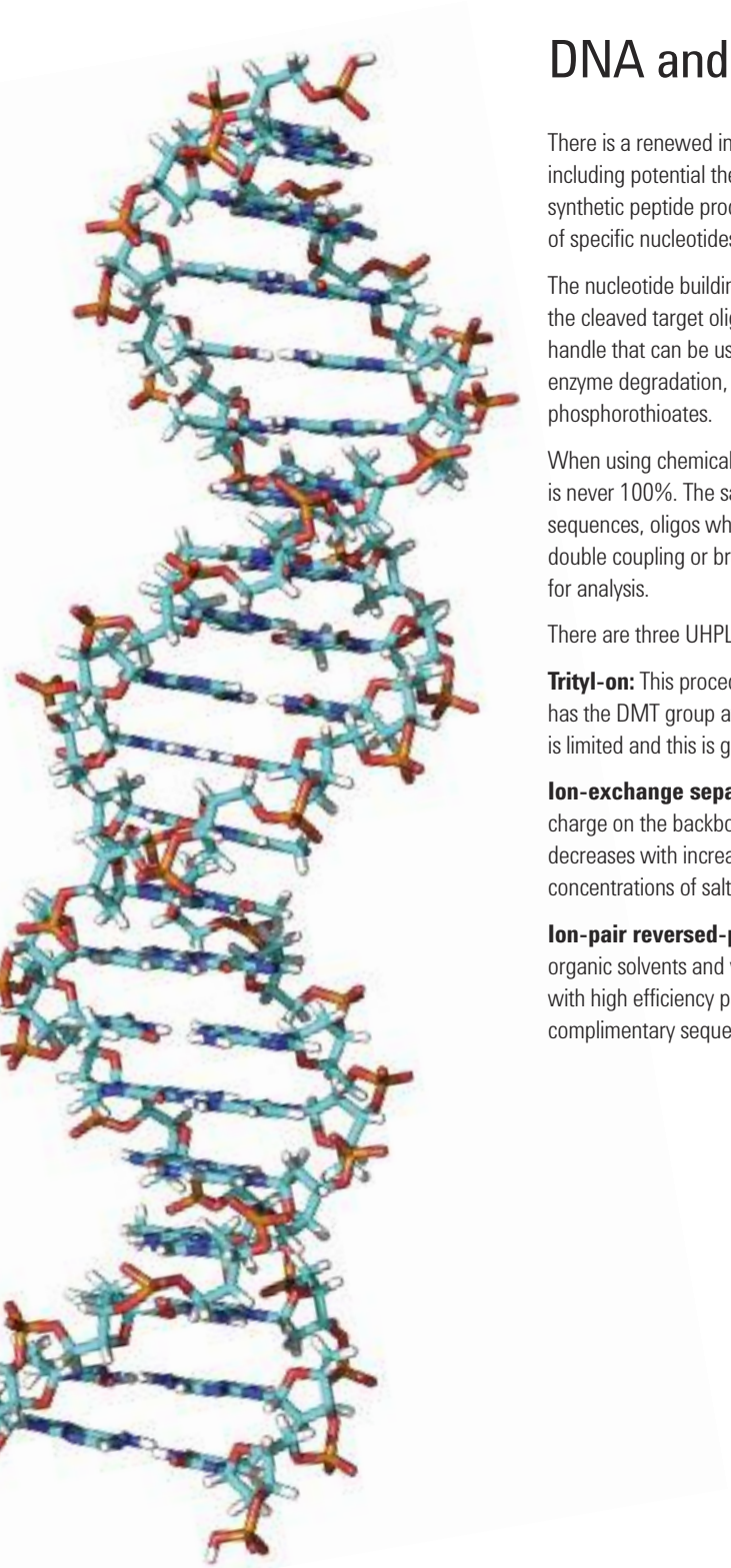
Flow Rate: Linear velocity 360 cm/hr



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

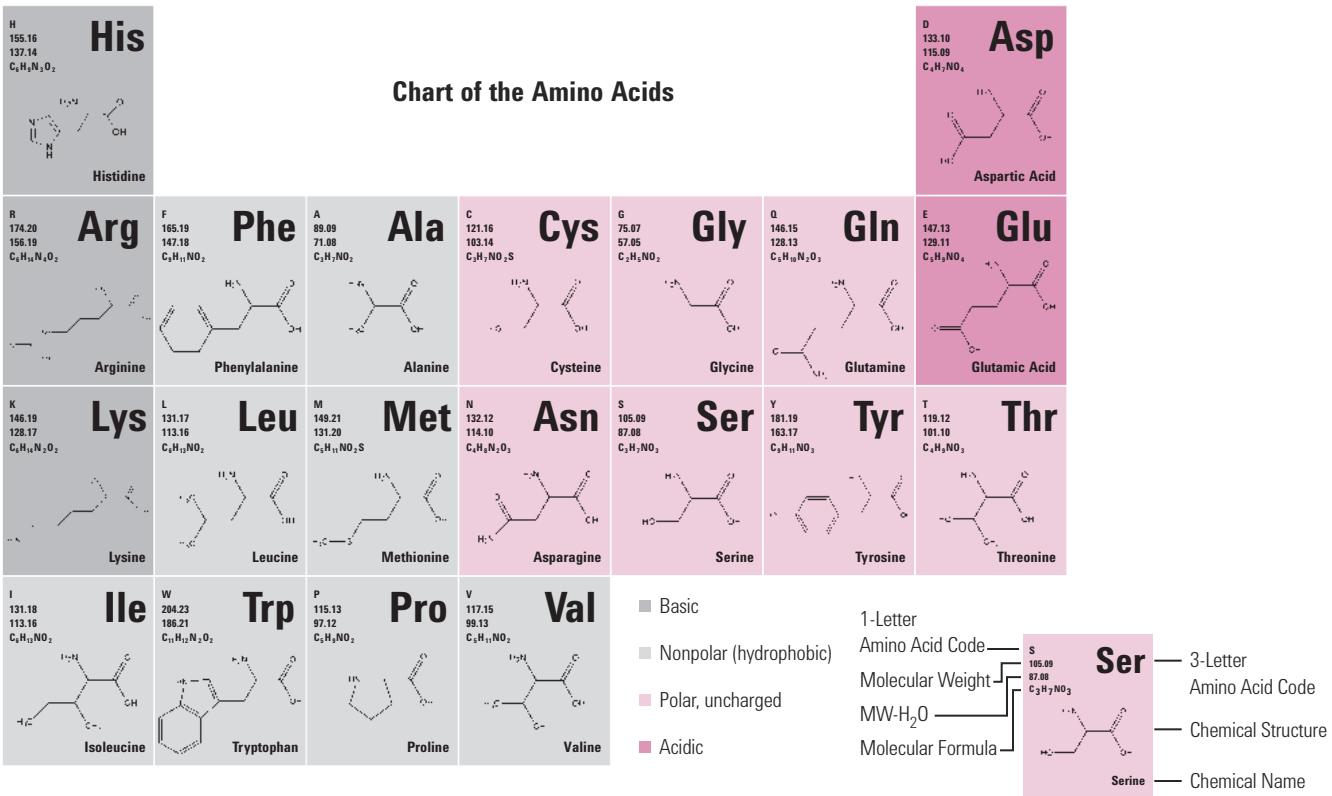
Agilent offers several good options for separation of amino acids, including the Agilent ZORBAX Eclipse AAA column which uses an updated protocol and is specially tested using amino acids. The ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. 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

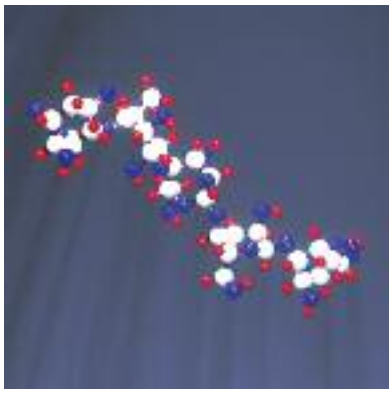
For more information on the ZORBAX Eclipse Plus C18 column, turn to page 248.

Chart of the Amino Acids



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 ü PL aquagel-OH 8 µm ü PL aquagel-OH 20 5 µm ü 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 ü PL aquagel-OH MIXED-H 8 µm ü 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	ü High resolution ü High capacity ü Relatively simple ü Sample concentrated on-column ü Small particle, 1.8 µm, for UHPLC separations ü Polymeric media for unsurpassed chemically and thermally stable	ü Denaturing conditions ü High efficiency silica columns cannot be cleaned using aggressive solvents when performing purifications
Ion-Exchange	ü Good recovery of biological activity ü High capacity ü Sample concentrated on-column	ü Limited MS compatibility due to presence of salts
Size Exclusion	ü Good recovery of biological activity ü Non-interactive technique with good sample recovery	ü No sample concentration ü Limited capacity
Affinity	ü Highly selective ü Good recovery of biological activity ü Sample concentrated on-column ü Often single step isolation	ü No sample concentration ü 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.

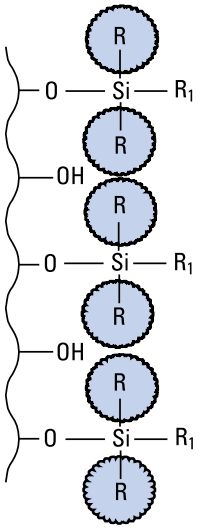
Reversed-Phase Column Selection

Application	Agilent Columns	Notes
Proteins and polypeptides	ZORBAX 300Å, 1.8 µm ü RRHD 300SB-C18 ü RRHD 300SB-C8 ü RRHD 300SB-C3 ü RRHD 300-Diphenyl ü 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 ü 300SB-C18 ü 300SB-C8 ü 300SB-C3 ü 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 ü 300SB-C18 ü 300SB-C8 ü 300SB-C3 ü 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 ü 100Å ü 300Å ü 1000Å ü 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

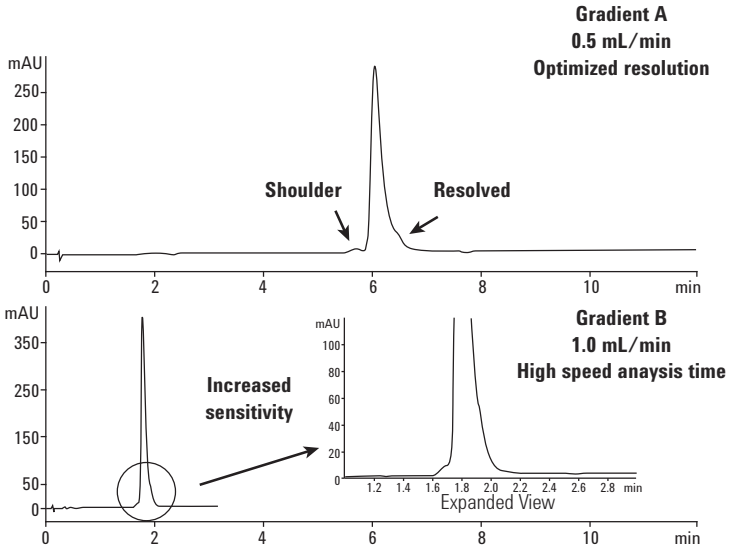
Mobile Phase: A: H_2O:IPA (98:2) + 0.1% TFA (v/v)
B: IPA:ACN:H_2O (70:20:10) + 0.1% TFA (v/v)

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

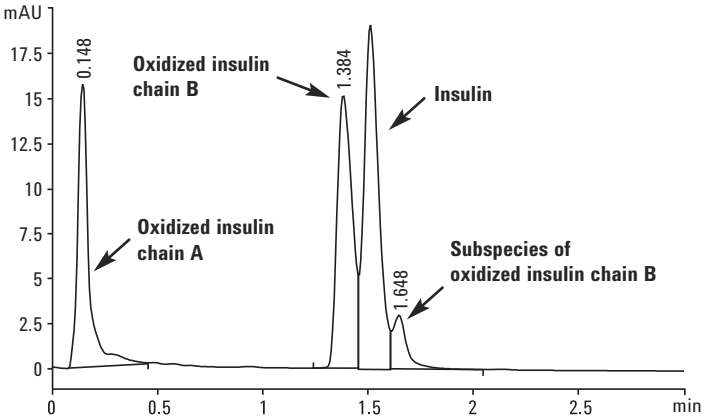
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

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

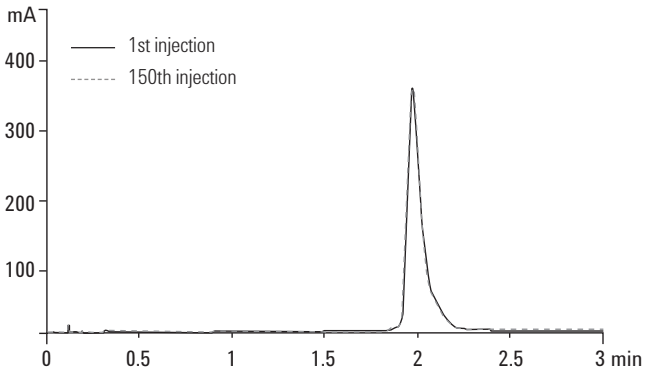
Mobile Phase: A: H_20:IPA (98.2), 0.1% TFA
B: IPA:ACN:H_20 (70:20:10), 0.1% TFA

Flow Rate: 1.0 mL/min

Temperature: 80 °C

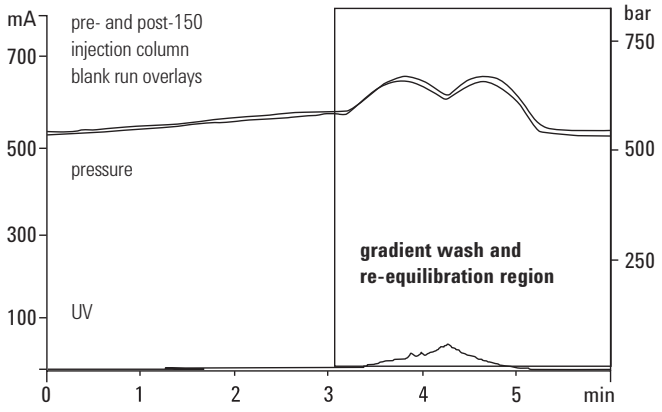
Detector: 1290 Infinity LC with diode array detector at 225 nm

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

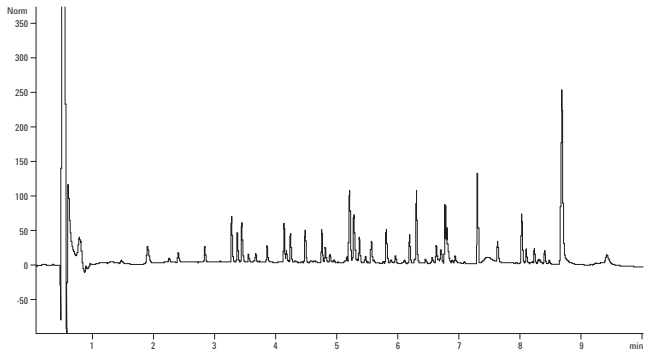
Flow Rate: 0.5 mL/min

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

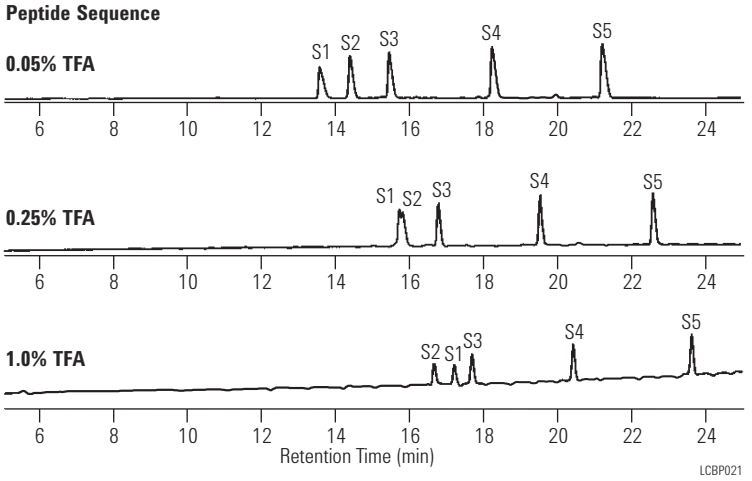
Flow Rate: 1.0 mL/min

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%)

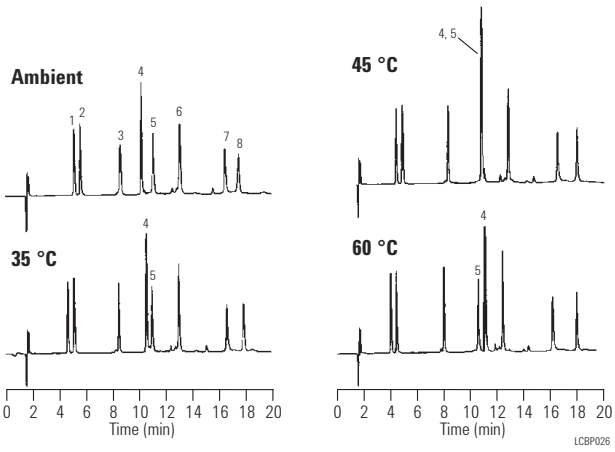
Flow Rate: 1.0 mL/min

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**

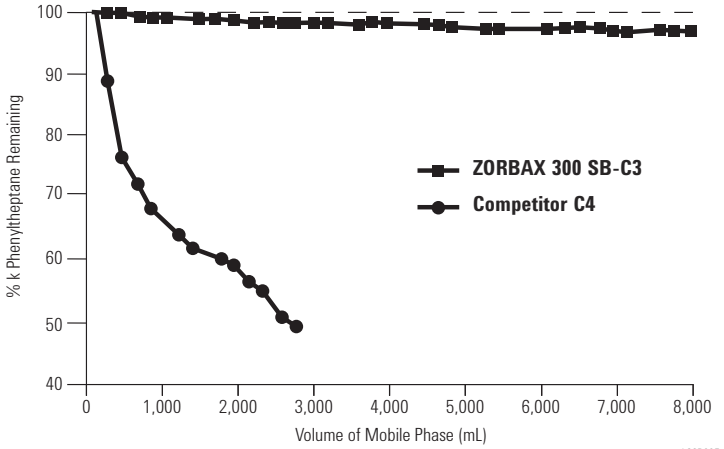
Short-chain ZORBAX 300SB-C3 is stable at low pH, high temperature

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

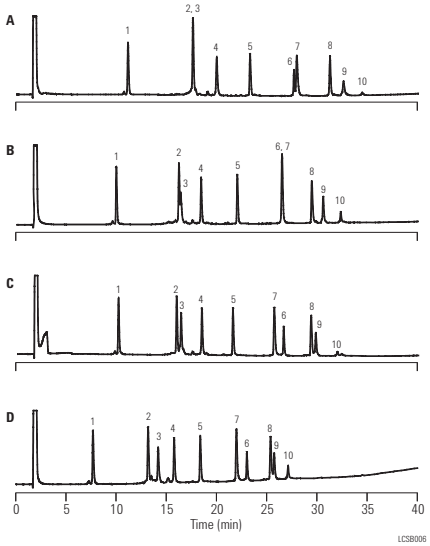
Column D: **ZORBAX 300SB-CN**
883995-905
4.6 x 150 mm, 5 µm

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)

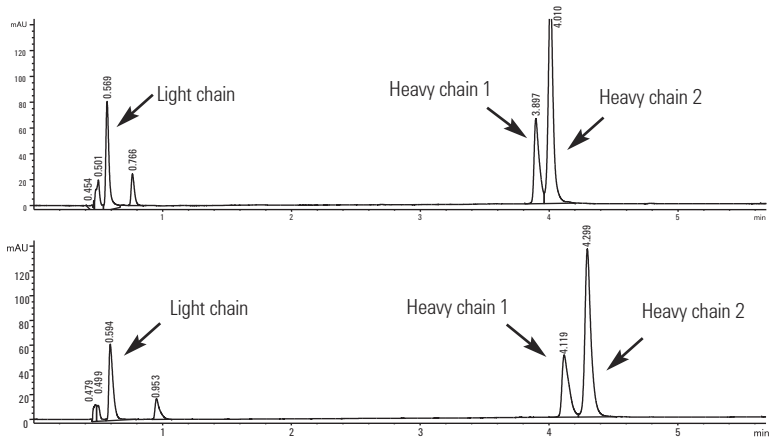
Sample Injection: 2 µL

Flow Rate: 0.5 mL/min

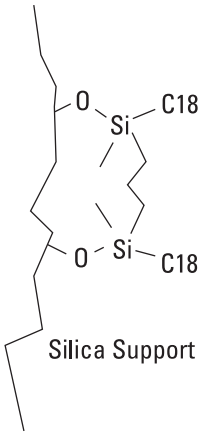
Gradient: 0 min-1%B, 2 min-20% B, 5 min-50% B

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

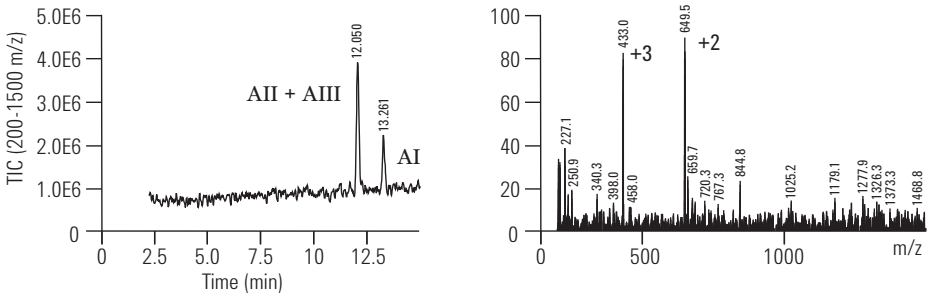
Column: **ZORBAX Extend-C18**
773700-902
2.1 x 150 mm, 5 µm

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

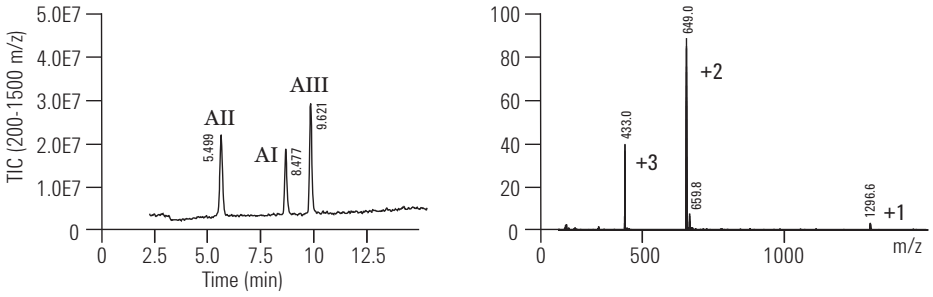
Flow Rate: 0.2 mL/min
Gradient: 15-50% B in 15 min
Temperature: 35 °C

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

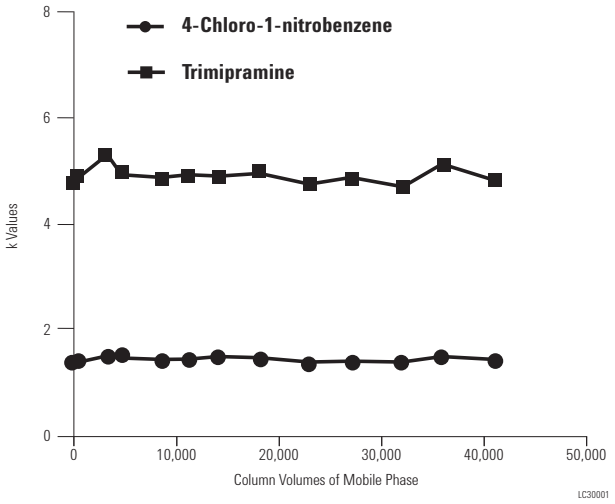
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

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

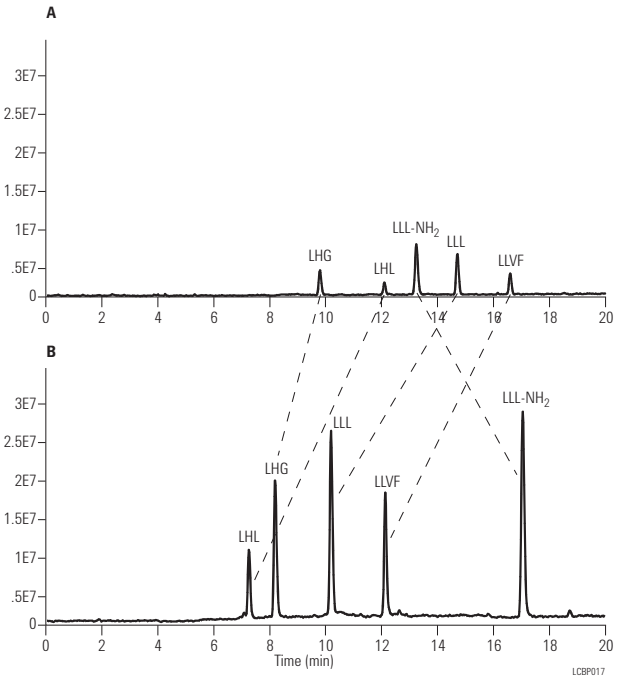
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

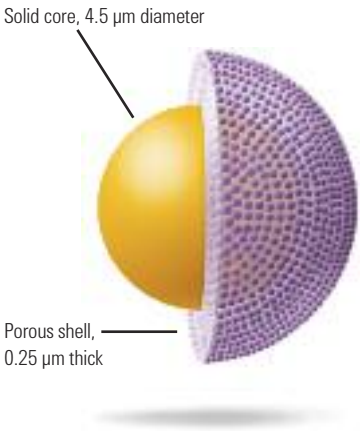
MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV
N_2 – 35 psi, 12 L/min, 300 °C
4 µL (50 ng each peptide)

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

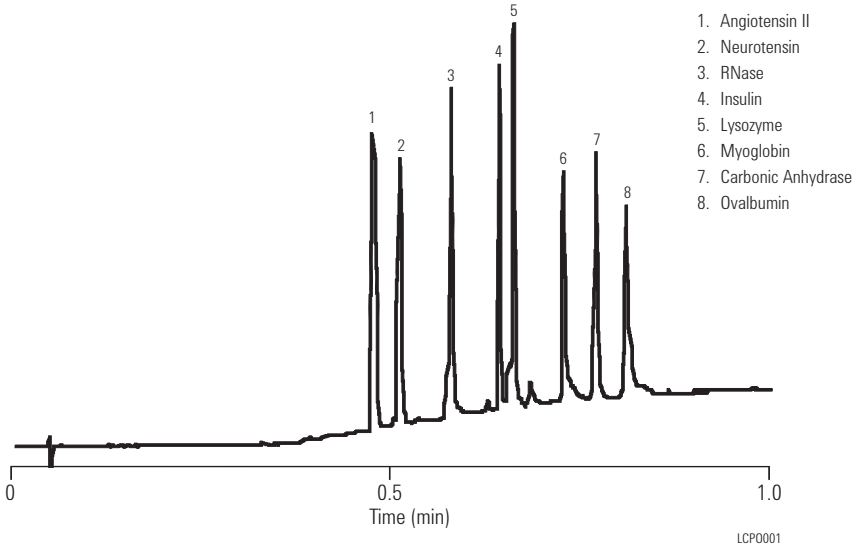
Specifications represent typical values only.

*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

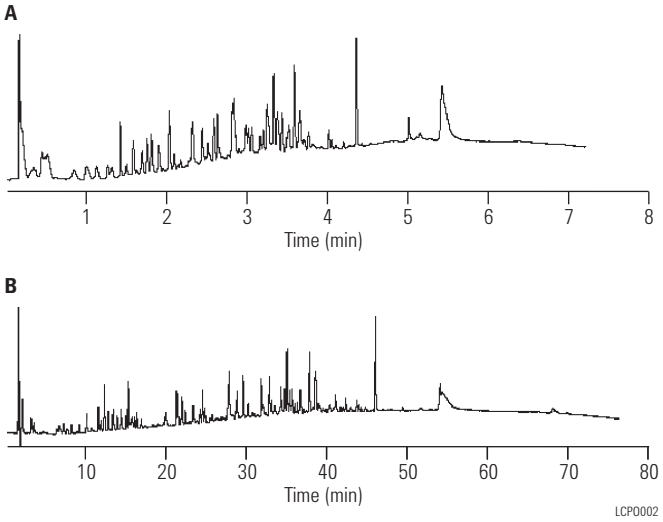
Mobile Phase: A: 95% H_2O, 5% ACN, 0.1% TFA
B: 5% H_2O, 95% ACN, 0.07% TFA

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

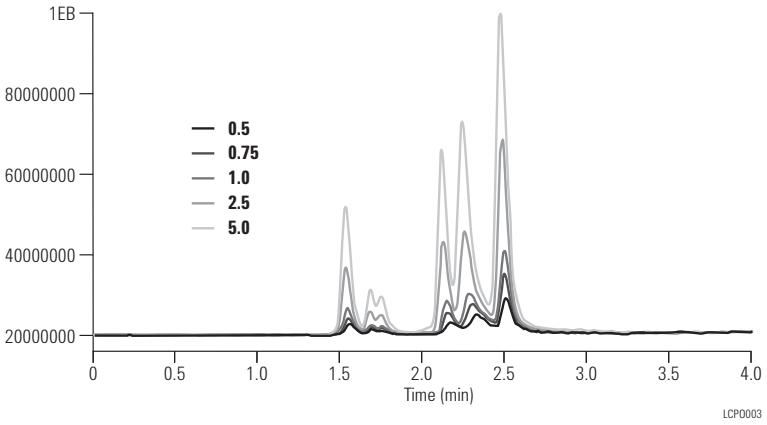
Flow Rate: 600 µL/min

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

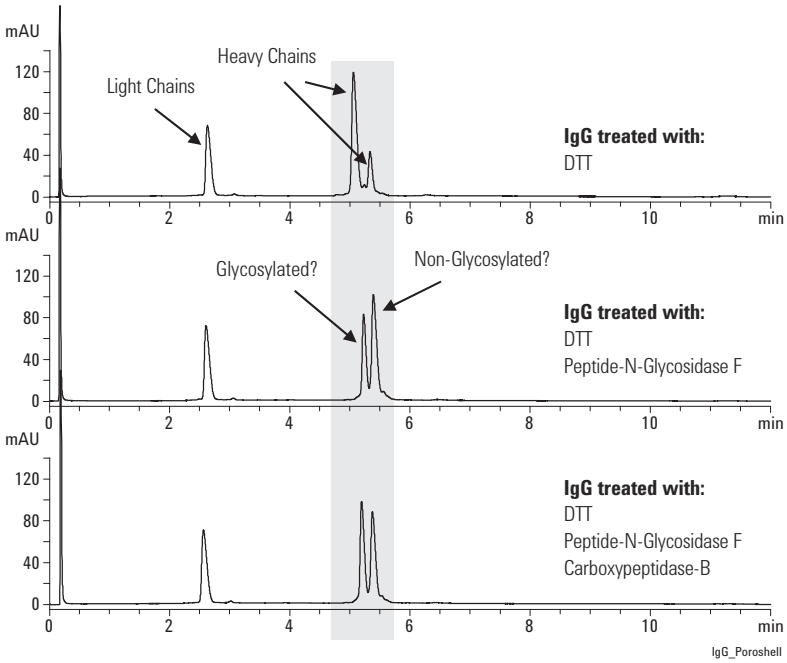
Flow Rate: 1.0 mL/min

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

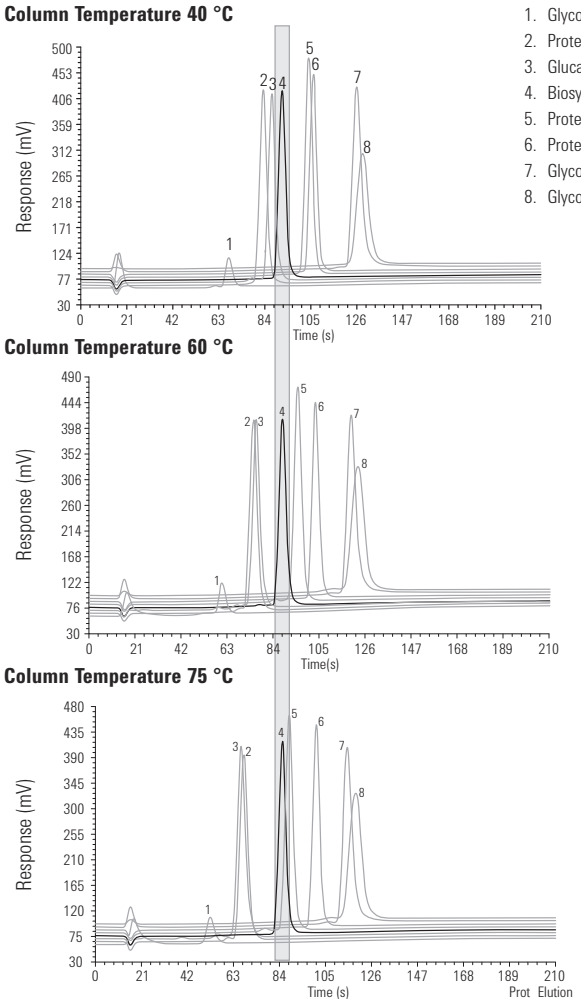
Column: **Poroshell 300SB-C8 660750-906 2.1 x 75 mm, 5 µm**

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.

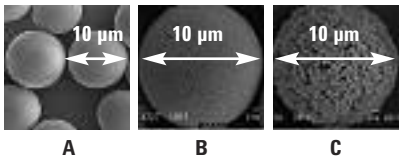
Poroshell 120

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

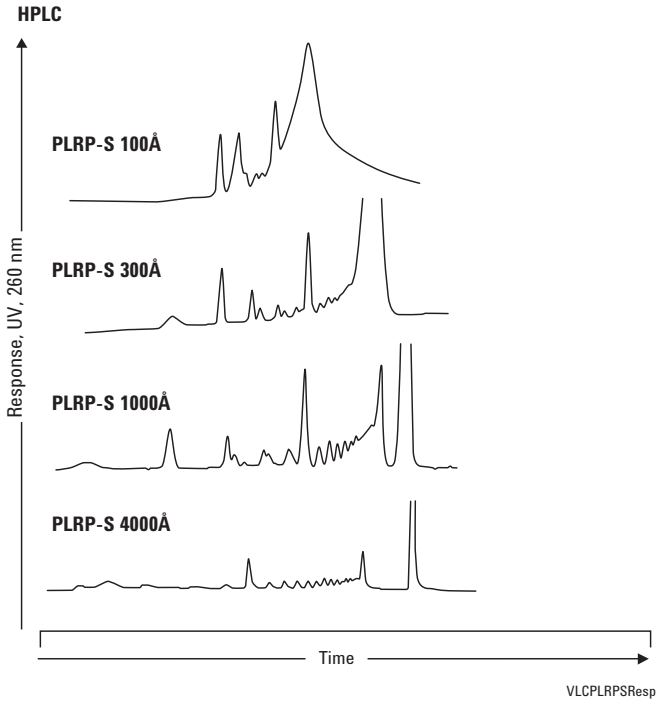
HPLC of 25 bp DNA ladder

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

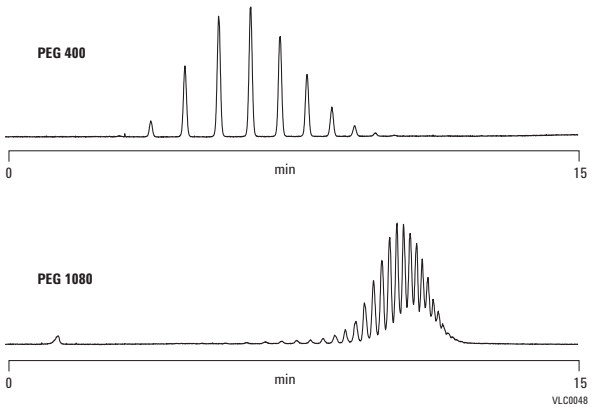
Gradient: 10-30% B in 12 min, held at 30% B for 3 min

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

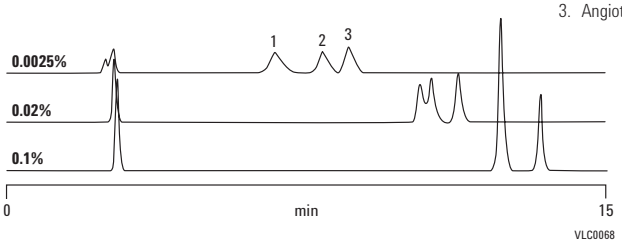
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

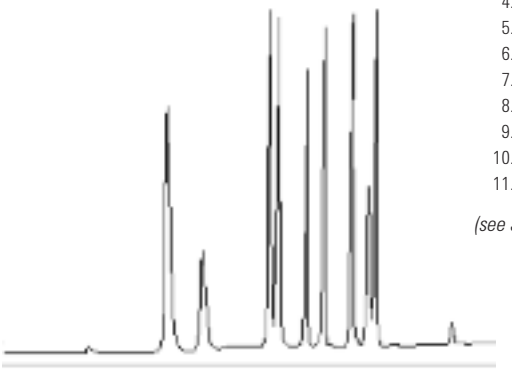
Mobile Phase: A: 0.1% TFA/1% 2-Propanol/Water
B: 0.1% TFA/1% 2-Propanol/ACN

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

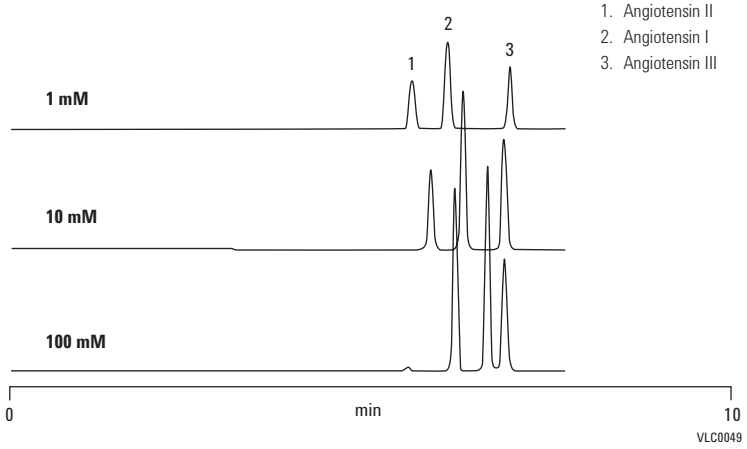
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

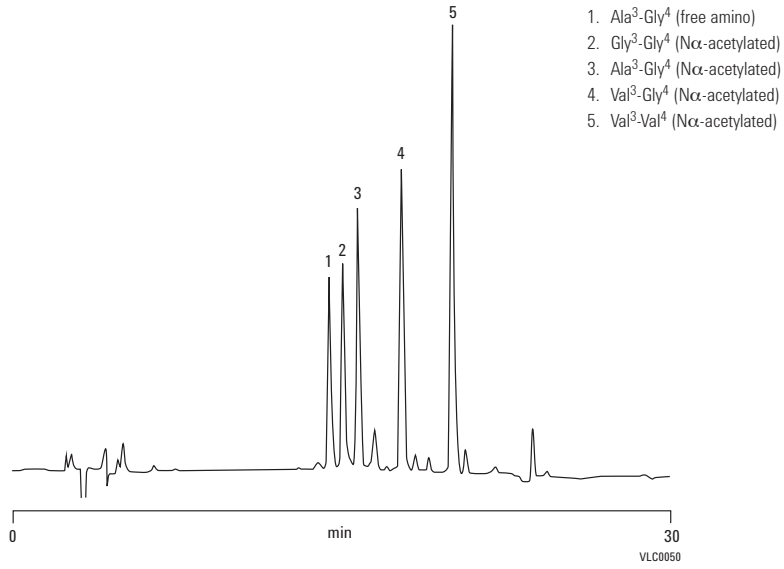
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

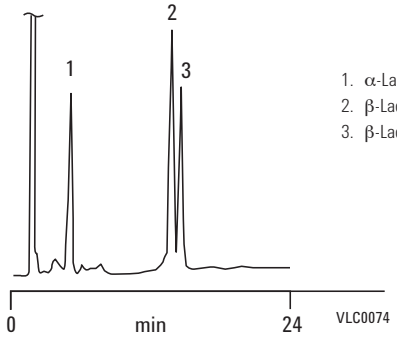
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

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

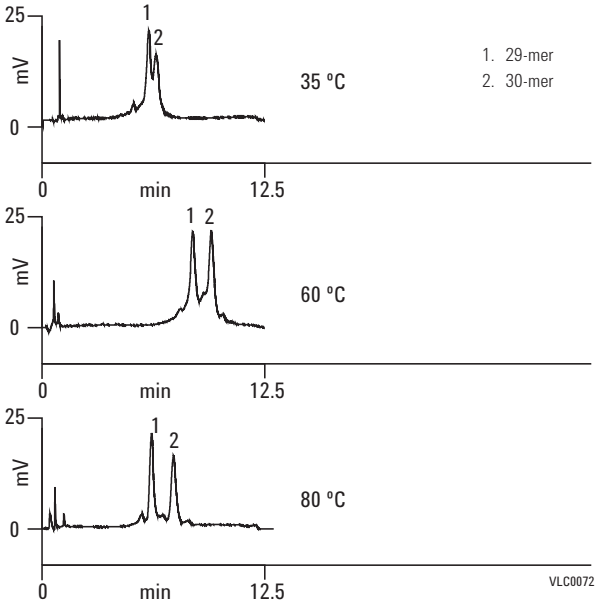
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

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

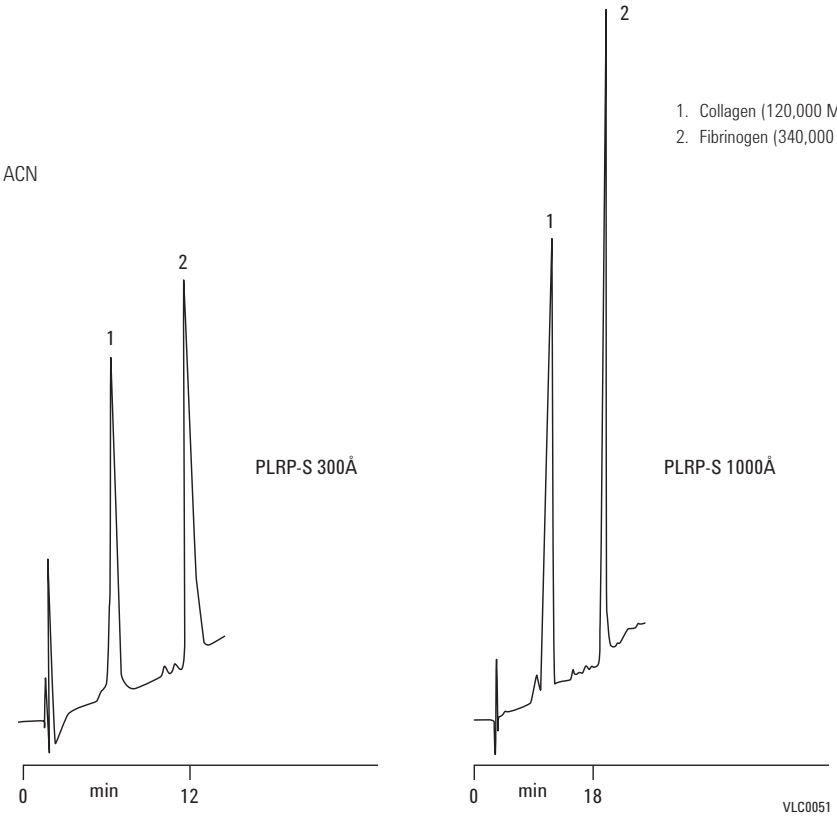
Column: **PLRP-S 1000Å**
PL1512-3802
4.6 x 150 mm, 8 µm

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



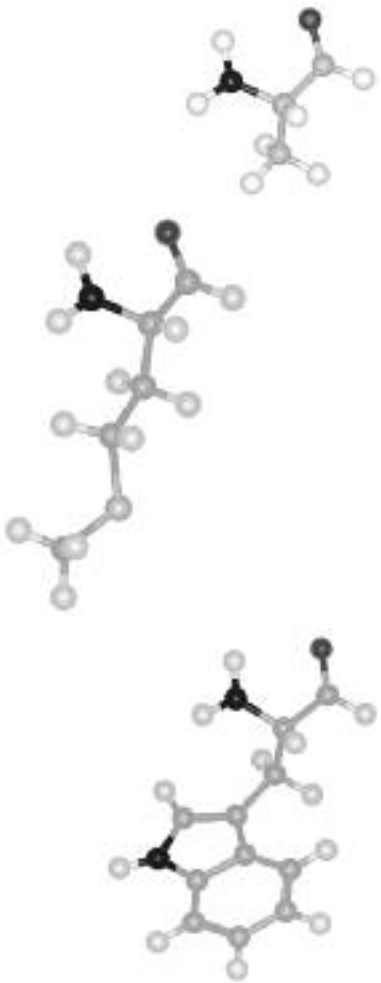
PLRP-S HPLC Columns

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

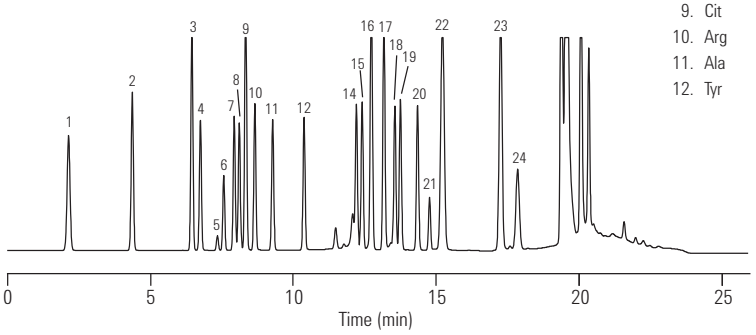
Mobile Phase: A: 40 mM Na_2HPO_4, pH 7.8
B: ACN:MeOH:Water, 45:45:10 v/v

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: Fluorescence

Sample: 24 Amino Acids



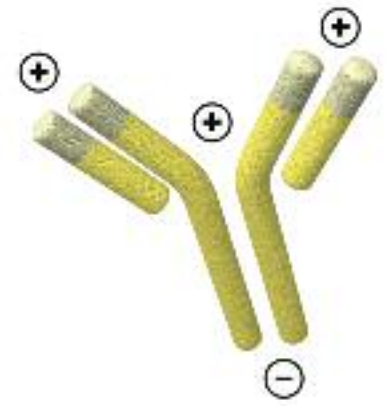
LCPAH01

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 ü 1000Å ü 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 ü 1000Å ü 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 ü Bio-Monolith QA ü Bio-Monolith DEAE ü Bio-Monolith SO_3 ü 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)

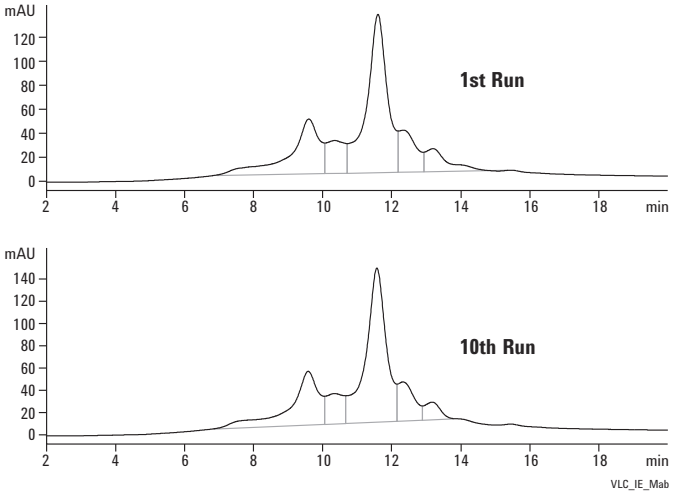
www.agilent.com/chem/library

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

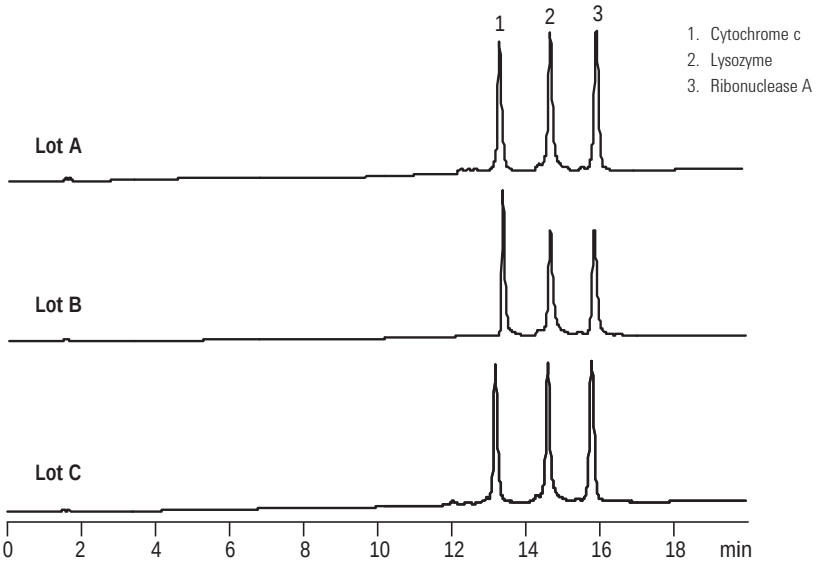
To provide a metal free flow path, Bio MAb PEEK columns are available.



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

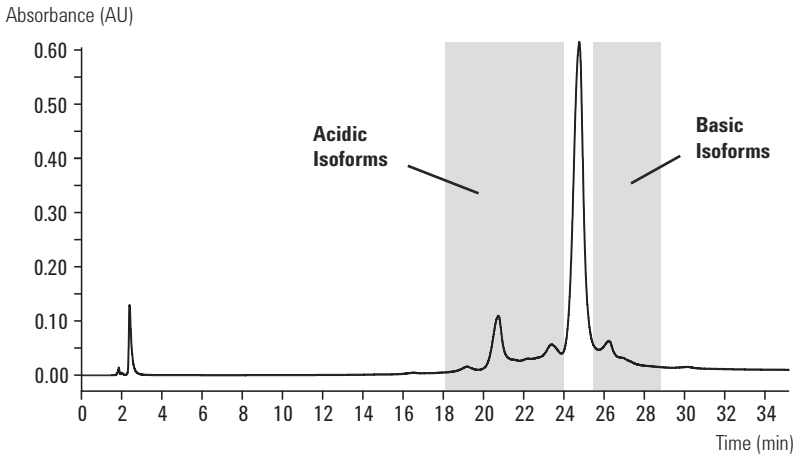
Column: **Bio MAb, PEEK 5190-2407 4.6 x 250 mm, 5 µm**

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

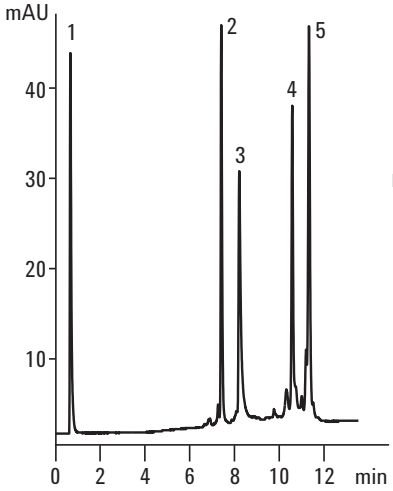
Buffer: 10 mM Phosphate, pH 6.0

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

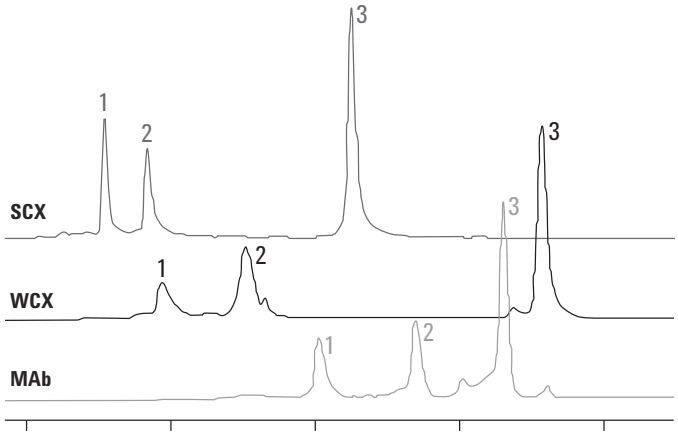


Illustration that Bio WCX, SCX and MAb columns are capable of producing protein separations

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

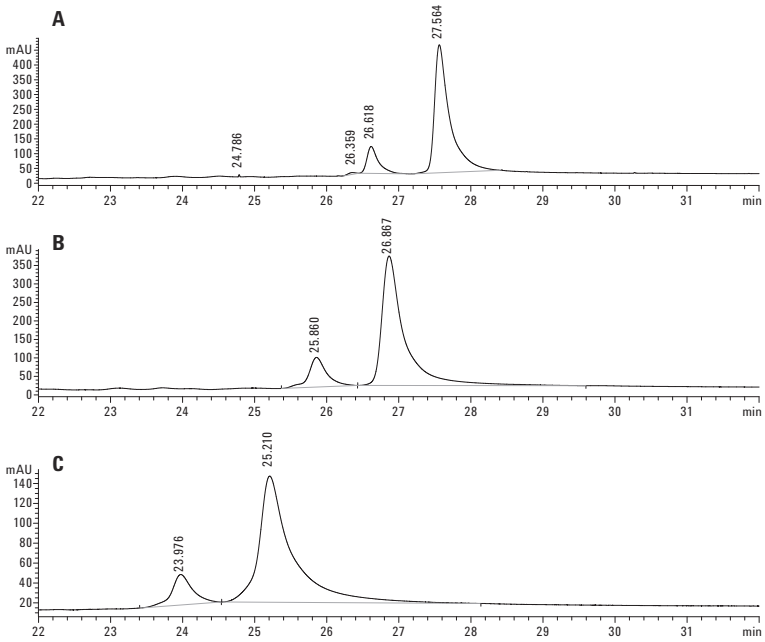
Flow Rate: 0.5 mL/min

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

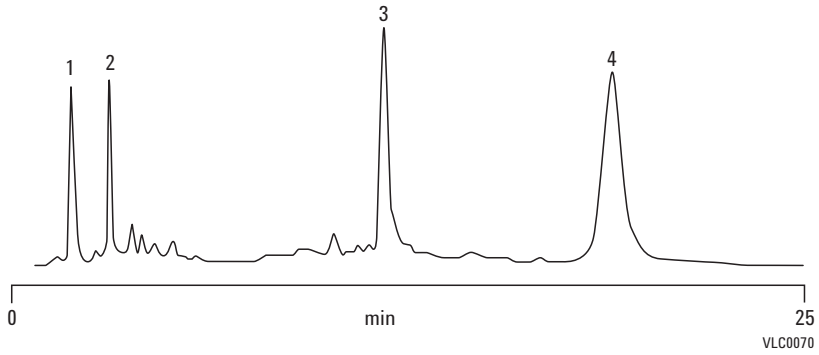
Mobile Phase: A: 10 mM Tris HCl pH 8
B: A+0.35 M NaCl pH 8

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



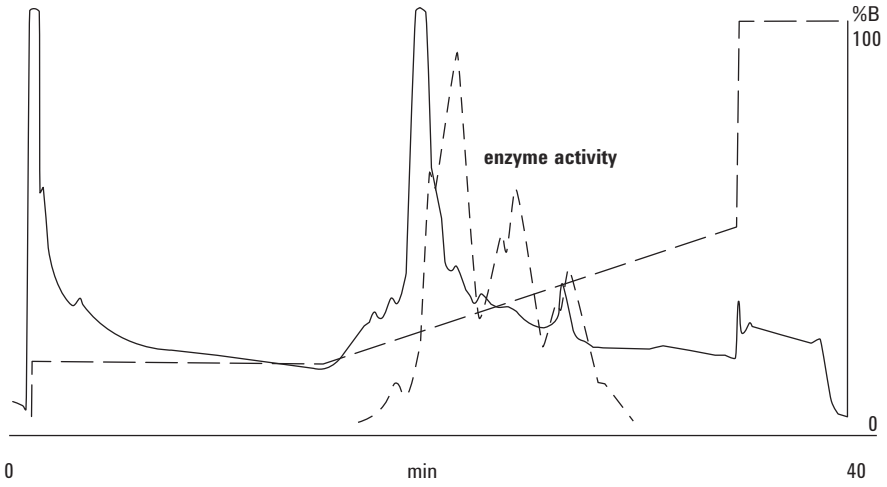
Analysis of choline kinase on PL-SAX 4000Å

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

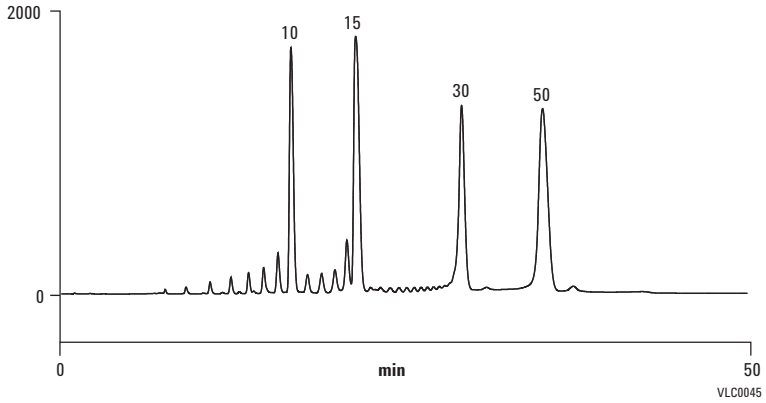
Mobile Phase: A: 7:93 v/v ACN: 0.1 M TEAA, pH 8.5
B: 7:93 v/v ACN: 0.1 M TEAA,
1 M ammonium chloride, pH 8.5

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

For more information

Buy online:
www.agilent.com/chem/store

Contact us:
www.agilent.com/chem/contactus

Printed in Canada October 31, 2012
5991-1059EN

Scan the QR code
with your smartphone
for more information

The Measure of Confidence

Agilent Technologies