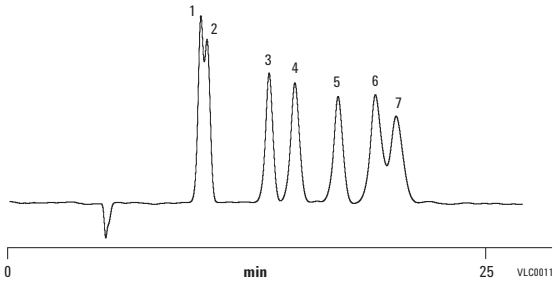
USP methods for sugar alcohols

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

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

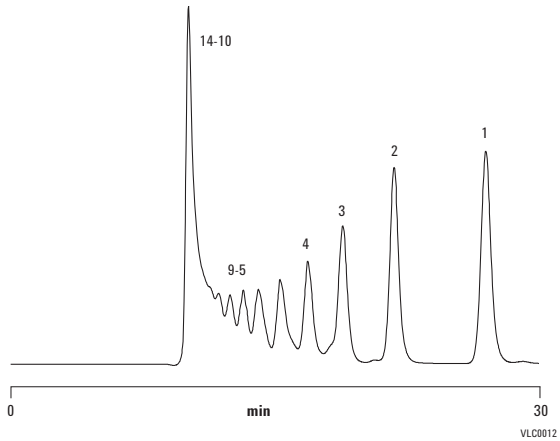


1. Iso-erythritol
2. Adonitol
3. Arabitol
4. Mannitol
5. Xylitol
6. Dulcitol
7. Sorbitol

Corn syrup, Hi-Plex

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

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



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

Analysis of sweeteners on Hi-Plex Ca columns

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

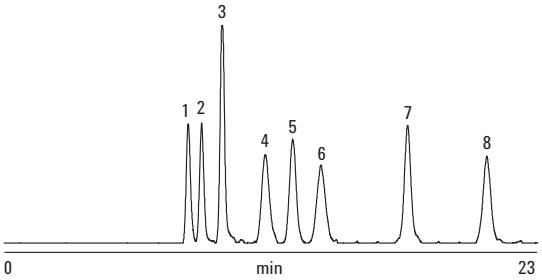
Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: ELSD

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



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

Analysis of carbohydates on Hi-Plex H columns

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

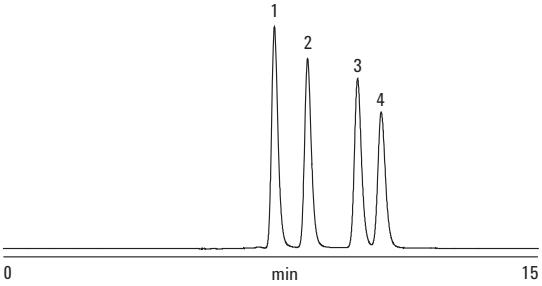
Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 70 °C

Detector: RI

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



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

Analysis of sugars with high sodium matrix

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

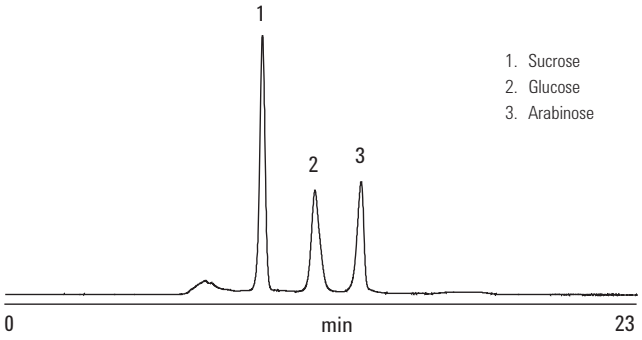
Mobile Phase: 0.015 M NaOH

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: RI

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



USP method for sorbitol

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

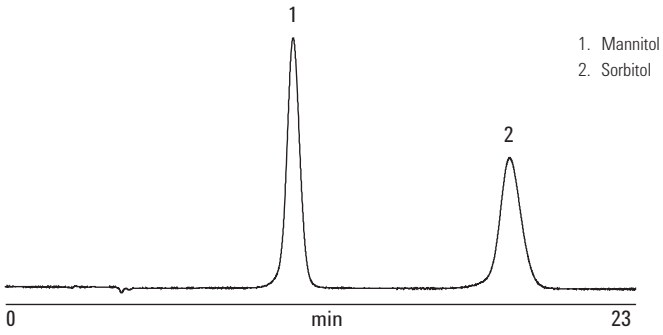
Mobile Phase: Water

Flow Rate: 0.7 mL/min

Temperature: 50 °C

Detector: RI

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



Hi-Plex Columns for Carbohydrate Analysis

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

Hi-Plex Guard Columns

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

Hi-Plex Guard Cartridges, 2/pk

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

Quick Guide to USP Designations for HPLC Columns

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

USP Designations

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

(Continued)

USP Designations

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

(Continued)

USP Designations

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

(Continued)

USP Designations

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

Oligo Solutions

StratoSpheres DNA Cartridges

StratoSpheres DNA Cartridges

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

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

StratoSpheres DNA Cartridges

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

TOP, TOP-DNA and TOP-RNA Cartridges

TOP, TOP-DNA and TOP-RNA Cartridges

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

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

TOP and TOP-DNA Cartridges

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

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

TIPS & TOOLS

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

TOP-RNA Cartridges

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

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

TOP, TOP-DNA and TOP-RNA Cartridges

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

Table of Contents

LC and LC/MS Columns for Biomolecules

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

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

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

What is a biomolecule?

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

In this section, we deal with the separation of:

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

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

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

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

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

What is a biocolumn?

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

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

Protein Separations

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

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

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

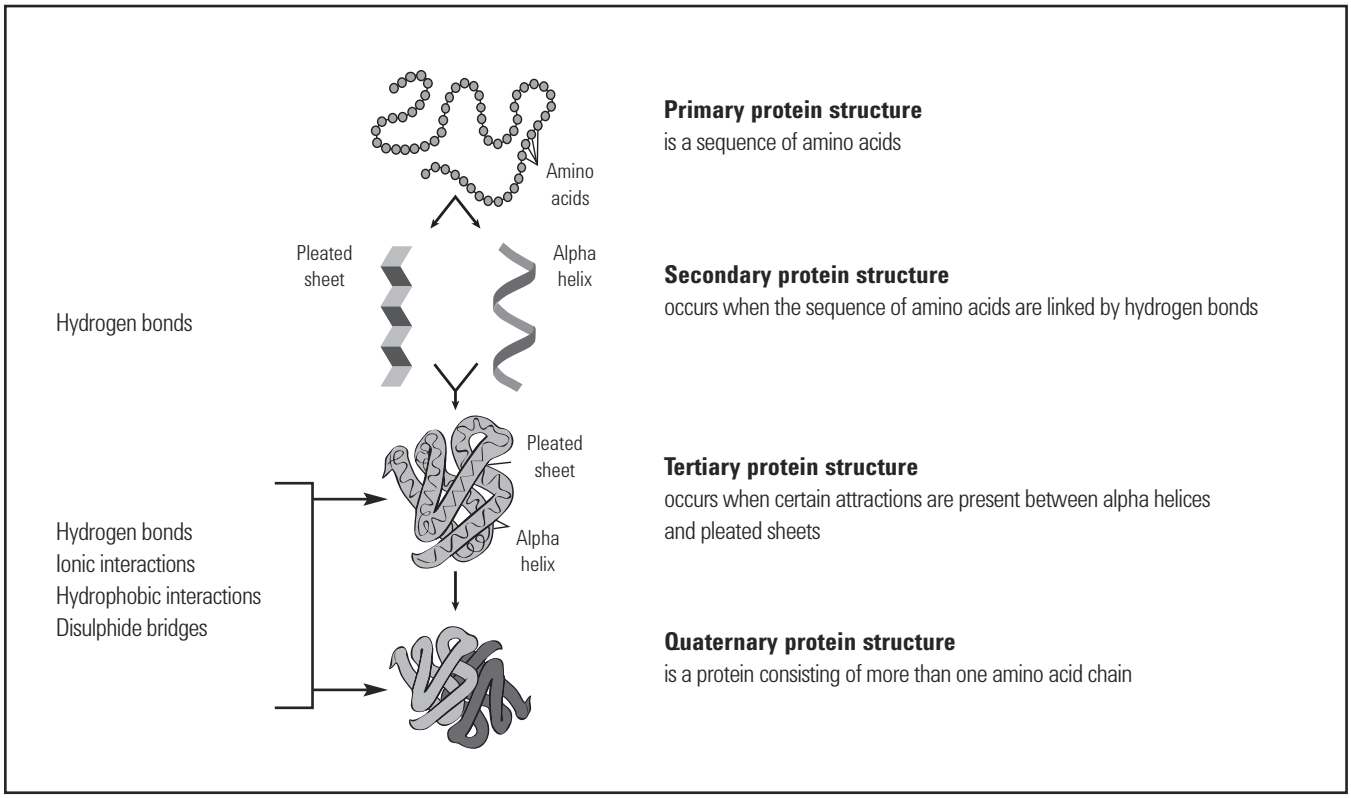


Figure 1. Schematic showing the various levels of protein structure.

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

Protein Column Selection Guide

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

Higher resolution of oxidation study

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

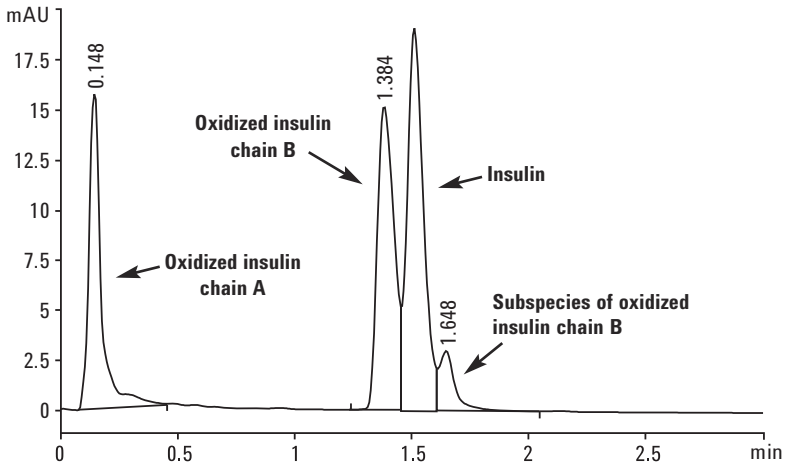
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

Flow Rate: 1.0 mL/min

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

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

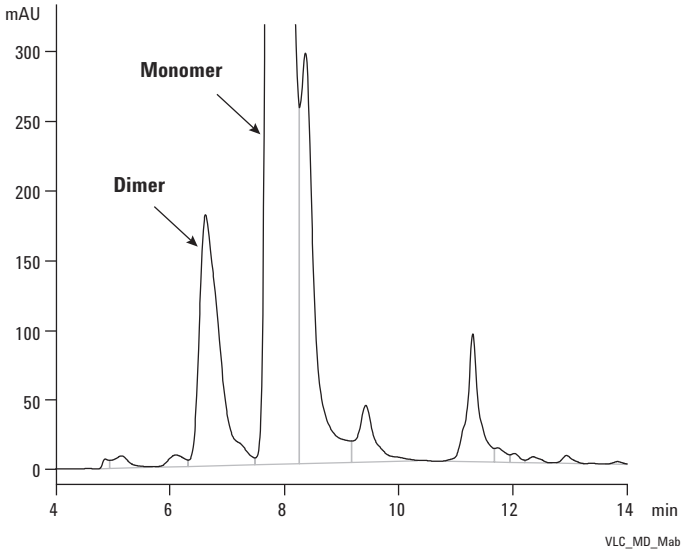
Sample: Insulin, insulin chain A and chain B, oxidized (bovinesigma, 1 mg/mL)



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

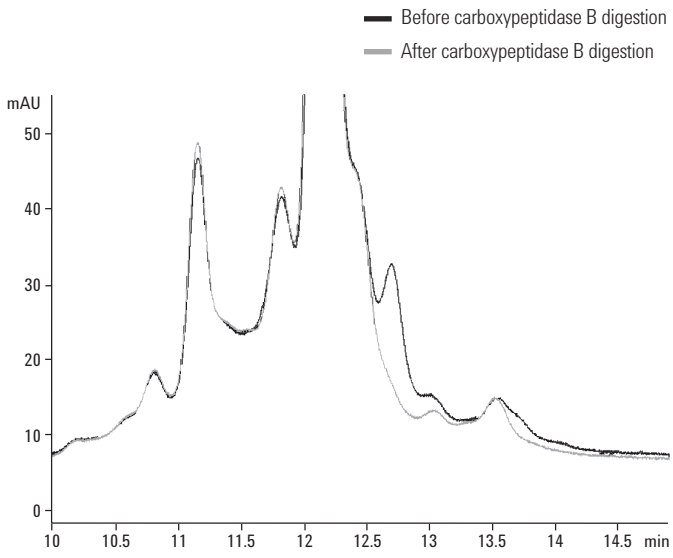
Intact MAb monomer and dimer separation

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



Separation of charge variants of human IgG1 with pH gradient

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



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

Peptide Separations

Peptide Mapping

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

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

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

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

TIPS & TOOLS

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

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

www.agilent.com/chem/library

Peptide Mapping Column Selection

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

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

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

Increased resolution for peptide mapping

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

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

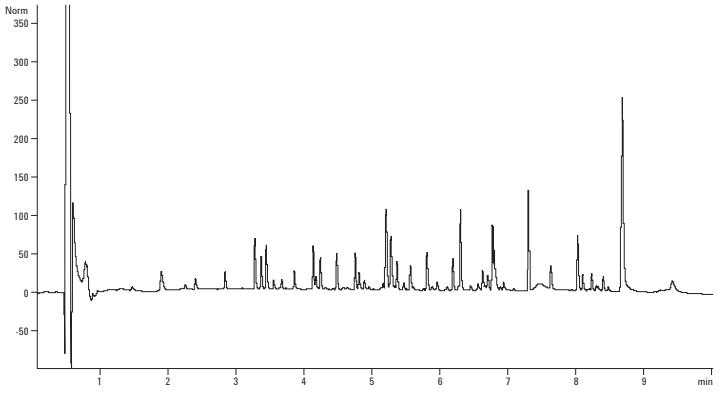
Flow Rate: 0.5 mL/min

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

Temperature: 50 °C

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

Sample: Enzymatic protein digest (MAb)



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

Separation of Natural and Synthetic Peptides

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

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

Natural and Synthetic Peptides Column Selection

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

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

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

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

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

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

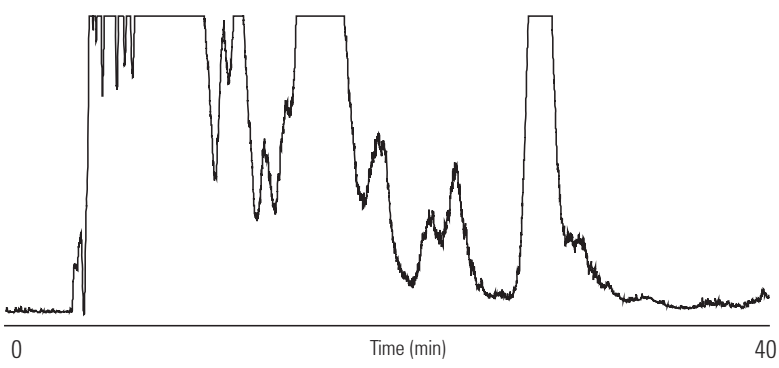
Preparative scale purification of Leuprolide by concentration overload

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

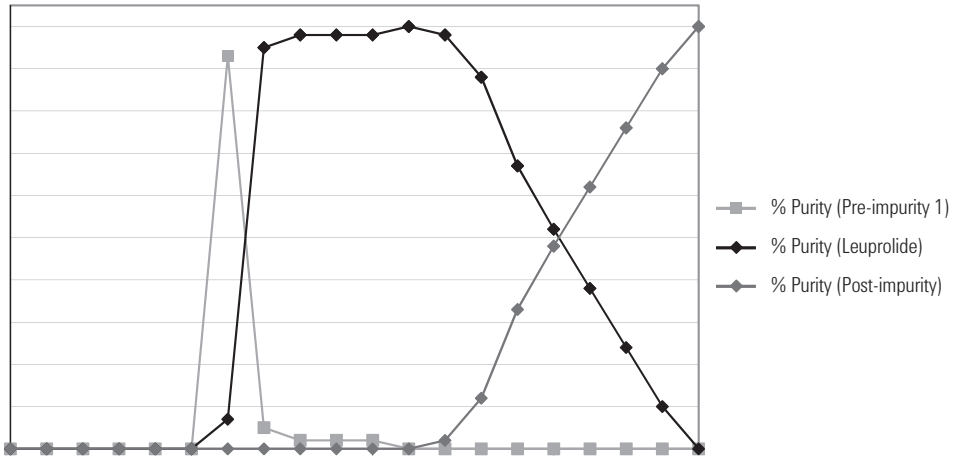
Bulk Media: **Load & Lock 4001 Column PCG93LL500X25**

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

Flow Rate: Linear velocity 360 cm/hr



Crude leuprolide separation of 30 mg on-column load.



Fraction analysis – the concentration overload purification.

DNA and RNA Oligonucleotide Separations

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

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

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

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

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

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

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

DNA and RNA Oligonucleotide Column Selection

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

TIPS & TOOLS

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

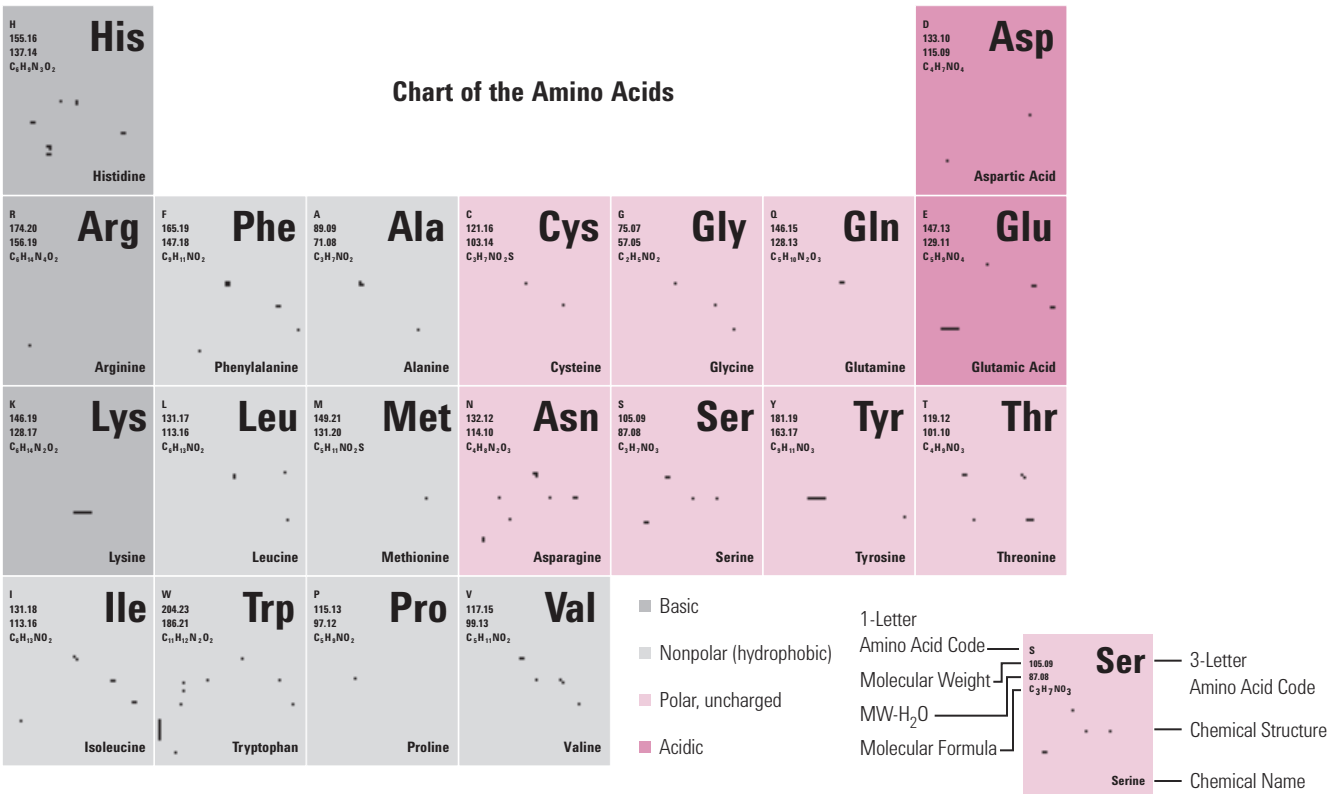
Amino Acid Analysis

Agilent offers several good options for separation of amino acids, including the Agilent ZORBAX Eclipse AAA column which uses an updated protocol and is specially tested using amino acids. The ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection to injection can be achieved in as little as 14 min (9 min analysis time) on shorter, 7.5 cm columns and 24 min (18 min analysis time) on the 15 cm column. Exceptional sensitivity (5 to 50 pmol with diode array or fluorescence detectors) and reliability are achieved using both OPA- and FMOC-derivatization chemistries in one fully automated procedure using the Agilent 1200 Infinity LC. The newer ZORBAX Eclipse Plus C18 column is also an excellent choice for amino acid separations.

ZORBAX Eclipse AAA Column Selection

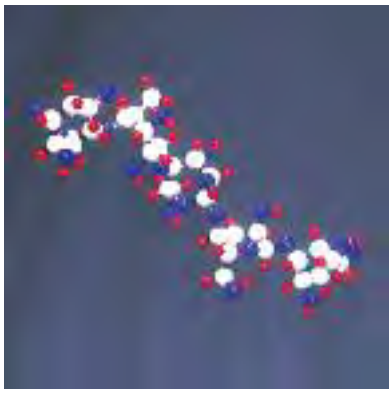
Application	Diameter x Length (mm)	Particle Size (µm)
Analytical routine sensitivity	4.6 x 150	5.0
Analytical routine sensitivity, high-resolution using FLD	4.6 x 150	3.5
Analytical routine sensitivity, high-throughput	4.6 x 75	3.5
Solvent Saver high sensitivity, high-resolution	3.0 x 150	3.5

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



Broad Distribution Biomolecules

Carbohydrates, Lipids and PEGs



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

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

UHPLC/HPLC Techniques

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

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

UHPLC/HPLC Techniques for Biomolecule Analysis

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

Reversed-Phase HPLC

Confidently perform high-resolution separations

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

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

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

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

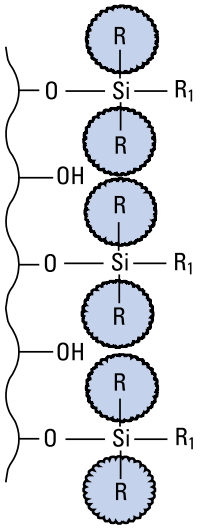
Reversed-Phase Column Selection

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

ZORBAX 300Å StableBond

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

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



Sterically Protected 300StableBond Bonded Phase

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp Limits*	pH Range*	Endcapped	Carbon Load
ZORBAX 300SB-C18	300Å	45 m^2/g	90 °C	1.0-8.0	No	2.8%
ZORBAX 300SB-C8	300Å	45 m^2/g	80 °C	1.0-8.0	No	1.5%
ZORBAX 300SB-C3	300Å	45 m^2/g	80 °C	1.0-8.0	No	1.1%
ZORBAX 300SB-CN	300Å	45 m^2/g	80 °C	1.0-8.0	No	1.2%
ZORBAX 300-Diphenyl	300Å	45 m^2/g	80 °C	1.0-8.0	Yes	1.9%

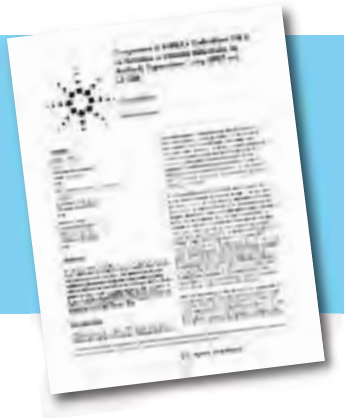
Specifications represent typical values only

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

TIPS & TOOLS

Further information can be found in the following publication:

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



Higher resolution of intact monoclonal antibody

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

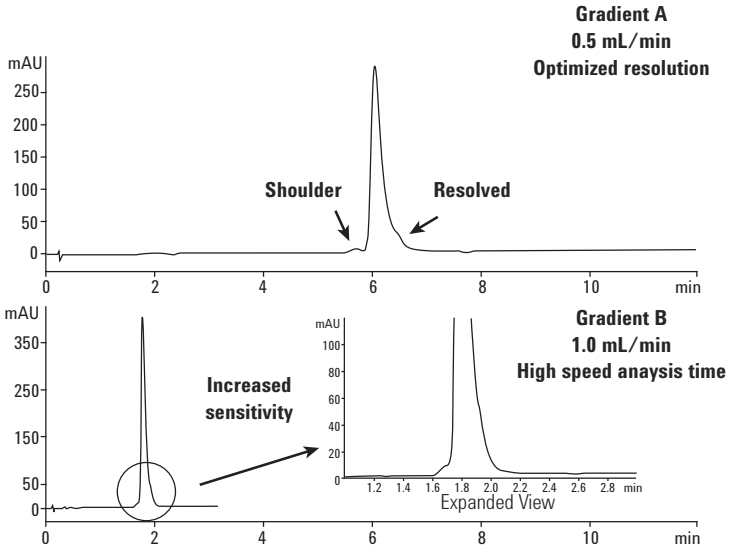
Mobile Phase: A: H_2O:IPA (98:2) + 0.1% TFA (v/v)
B: IPA:ACN:H_2O (70:20:10) + 0.1% TFA (v/v)

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

Gradient: Multi-segmented and linear elution

Temperature: 80 °C

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



Higher resolution of oxidation study

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

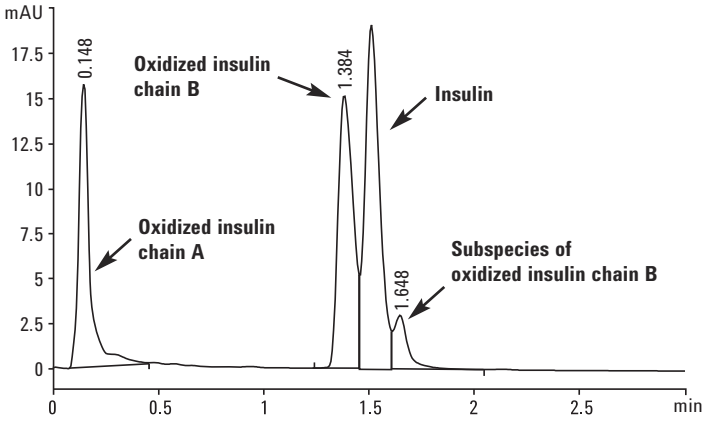
Mobile Phase: A: 0.1% TFA
B: 0.01% TFA + 80% ACN

Flow Rate: 1.0 mL/min

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

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

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



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

TIPS & TOOLS

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

Improved reproducibility of monoclonal antibodies

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

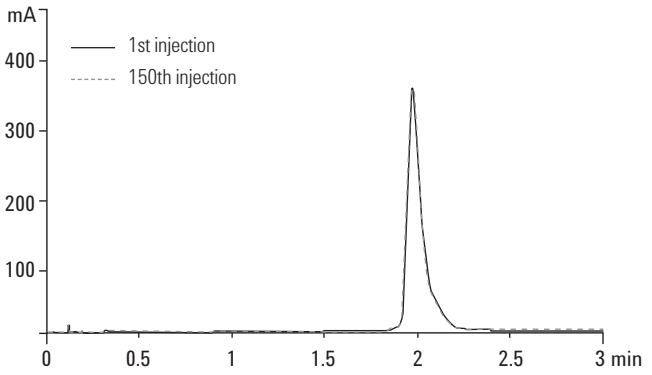
Mobile Phase: A: H_2O:IPA (98.2), 0.1% TFA
B: IPA:ACN:H_2O (70:20:10), 0.1% TFA

Flow Rate: 1.0 mL/min

Temperature: 80 °C

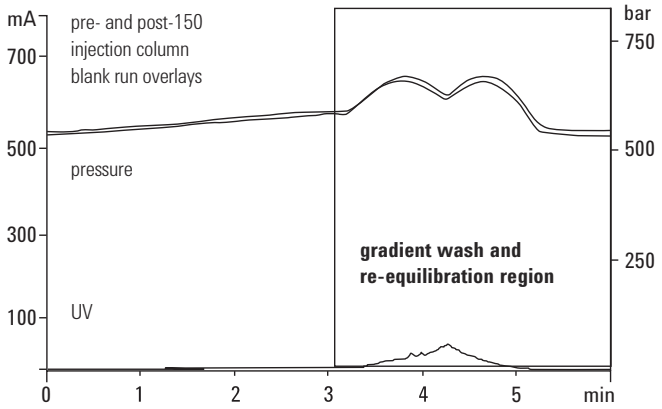
Detector: 1290 Infinity LC with diode array detector at 225 nm

Sample: MAb



Gradient timescale

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



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

Increased resolution for peptide mapping

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

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

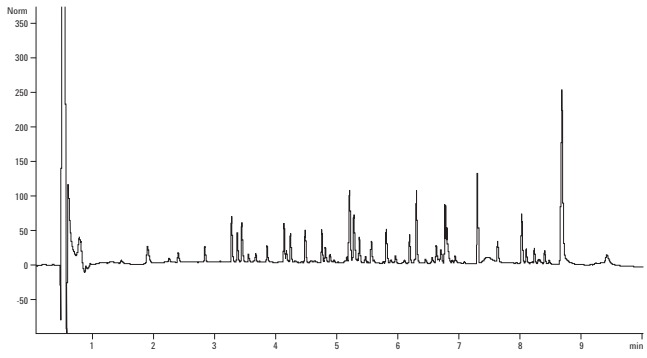
Flow Rate: 0.5 mL/min

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

Temperature: 50 °C

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

Sample: Enzymatic protein digest (MAb)



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

Peptides: Effect of TFA concentration

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

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

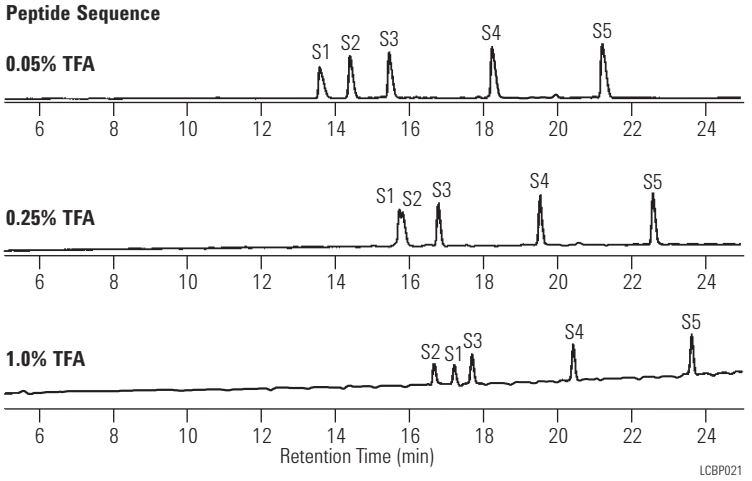
Flow Rate: 1.0 mL/min

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

Temperature: 40 °C

Detector: UV 254 nm

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



Peptides/proteins: Effect of elevated temperature

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

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

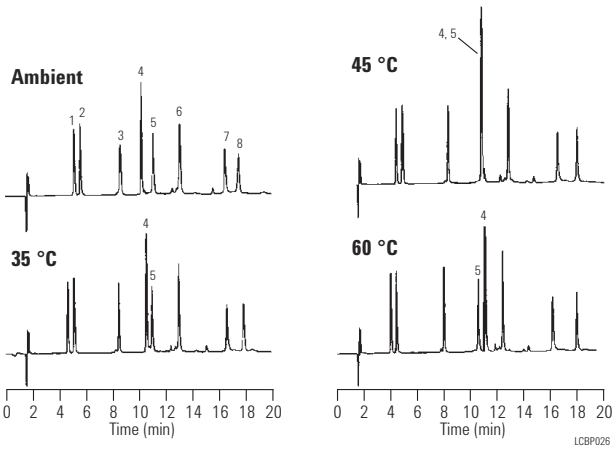
Flow Rate: 1.0 mL/min

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

Temperature: Ambient – 60 °C

Detector: UV 215 nm

Sample: Polypeptides



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

TIPS & TOOLS

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

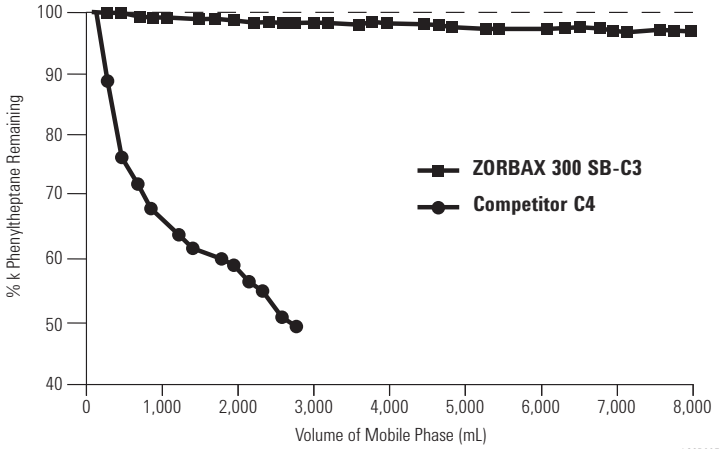
Short-chain ZORBAX 300SB-C3 is stable at low pH, high temperature

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

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

Flow Rate: 1.0 mL/min

Temperature: 60 °C



Four different 300SB bonded phases optimize separation of large polypeptides

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

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

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

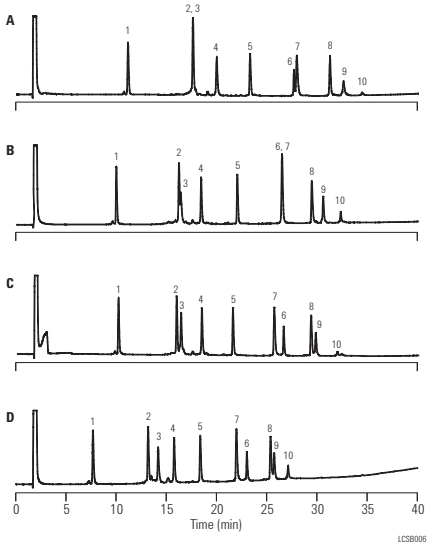
Column D: **ZORBAX 300SB-CN**
883995-905
4.6 x 150 mm, 5 µm

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

Flow Rate: 1.0 mL/min

Temperature: 60 °C

Sample: 3 µg each protein



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

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

ZORBAX 300Å StableBond

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
Standard Columns (no special hardware required)								
	Semi-Preparative	9.4 x 250	5	880995-202	880995-206	880995-205	880995-209	
	Analytical	4.6 x 250	5	880995-902	880995-906	880995-905	880995-909	
	Analytical	4.6 x 150	5	883995-902	883995-906	883995-905	883995-909	
	Analytical	4.6 x 50	5	860950-902	860950-906	860950-905	860950-909	
	Rapid Resolution	4.6 x 150	3.5	863973-902	863973-906	863973-905	863973-909	
	Rapid Resolution	4.6 x 100	3.5	861973-902	861973-906			
	Rapid Resolution	4.6 x 50	3.5	865973-902	865973-906	865973-905	865973-909	
	Solvent Saver Plus	3.0 x 150	3.5	863974-302	863974-306		863974-309	
	Solvent Saver Plus	3.0 x 100	3.5		861973-306			
	Narrow Bore	2.1 x 250	5	881750-902				
	Narrow Bore	2.1 x 150	5	883750-902	883750-906	883750-905	883750-909	
	Narrow Bore RR	2.1 x 150	3.5		863750-906			
	Narrow Bore RR	2.1 x 100	3.5	861775-902	861775-906			
	Narrow Bore RR	2.1 x 50	3.5	865750-902	865750-906			
	Narrow Bore RRHD	2.1 x 100	1.8	858750-902	858750-906		858750-909	858750-944
	Narrow Bore RRHD	2.1 x 50	1.8	857750-902	857750-906		857750-909	857750-944
	MicroBore	1.0 x 250	5	861630-902				
	MicroBore RR	1.0 x 150	3.5	863630-902	863630-906			
	MicroBore RR	1.0 x 50	3.5	865630-902	865630-906			
	MicroBore Guard, 3/pk	1.0 x 17	5	5185-5920	5185-5920			
P	Guard Cartridge, 2/pk	9.4 x 15	7	820675-124	820675-124	820675-124	820675-124	
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-921	820950-918	820950-923	820950-924	
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-918	821125-918	821125-924	821125-924	
P	Guard Hardware Kit			840140-901	840140-901	840140-901	840140-901	
ZGC	Guard Hardware Kit			820999-901	820999-901	820999-901	820999-901	

(Continued)

ZORBAX 300Å StableBond

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56	300-Diphenyl USP L11
PrepHT Cartridge Columns (require endfittings kit 820400-901)								
PL	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109	
PL	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109	
PL	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909	
PL	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909	
PL	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909	
PL	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901	
PL	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924	
PL	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901	
Capillary Glass-lined Columns								
	Capillary	0.5 x 250	5	5064-8266				
	Capillary	0.5 x 150	5	5064-8264				
	Capillary	0.5 x 35	5	5064-8294				
	Capillary RR	0.5 x 150	3.5	5064-8268				
	Capillary RR	0.5 x 35	3.5	5065-4459				
	Capillary	0.3 x 250	5	5064-8265				
	Capillary	0.3 x 150	5	5064-8263				
	Capillary	0.3 x 35	5	5064-8295				
	Capillary RR	0.3 x 150	3.5	5064-8267	5065-4460			
	Capillary RR	0.3 x 100	3.5	5064-8259	5065-4461			
	Capillary RR	0.3 x 35	3.5	5064-8270	5065-4462			
	Capillary RR	0.3 x 50	3.5	5064-8300	5065-4463			
Nano Columns (PEEK fused silica)								
	Nano RR	0.1 x 150	3.5	5065-9910				
	Nano RR	0.075 x 150	3.5	5065-9911				
	Nano RR	0.075 x 50	3.5	5065-9924	5065-9923			
	Trap/Guard, 5/pk	0.3 x 5	5	5065-9913	5065-9914			
	Trap/Guard Hardware kit			5065-9915	5065-9915			

ZORBAX RRHD 300-Diphenyl

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

The diphenyl column can be used for:

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

The ZORBAX RRHD 300-Diphenyl provides:

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

Column Specifications

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

Specifications represent typical values only

Fast separation of reduced monoclonal antibody

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

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

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

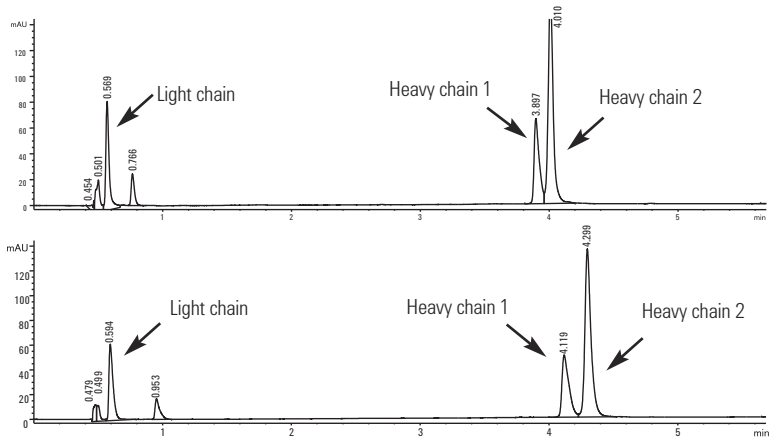
Sample Injection: 2 µL

Flow Rate: 0.5 mL/min

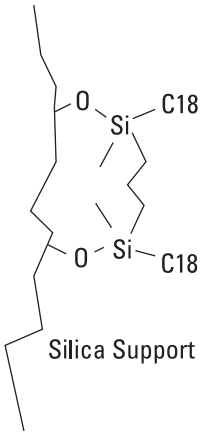
Gradient: 0 min-1%B, 2 min-20% B, 5 min-50% B

Temperature: 74 °C

Detector: UV, 280



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



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

ZORBAX 300Å Extend-C18

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

Agilent ZORBAX 300Å Extend-C18 is a wide-pore HPLC column for high efficiency separations of peptides from pH 2-11.5. The unique, bidentate bonded phase provides excellent lifetime and reproducibility at high and low pH. At high pH, retention and selectivity of peptides and polypeptides can change dramatically as a result of changes in charge on molecules. Excellent recoveries of hydrophobic polypeptides have been achieved at room temperature and high pH. LC/MS sensitivity of peptides and polypeptides can also be improved at high pH using a simple ammonium-hydroxide-containing mobile phase.

Column Specifications

Bonded Phase	Pore Size	Surface Area	Temp. Limits*	pH Range	Endcapped	Carbon Load
ZORBAX 300Å Extend-C18	300Å	45 m^2/g	60 °C	2.0-11.5	Double	4%

Specifications represent typical values only.

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

TIPS & TOOLS

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

LC/MS analysis of angiotensin on Extend-C18

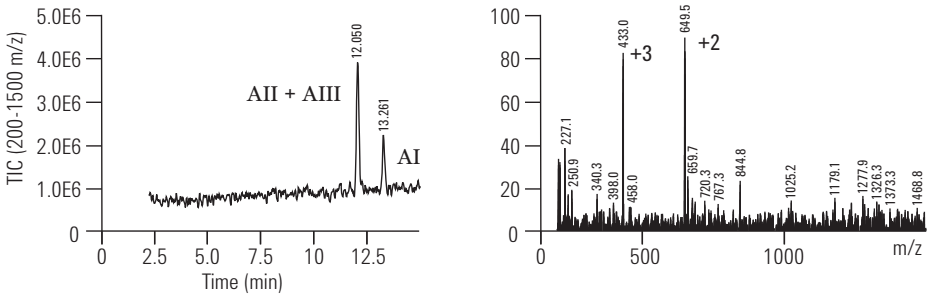
Column: **ZORBAX Extend-C18**
773700-902
2.1 x 150 mm, 5 µm

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

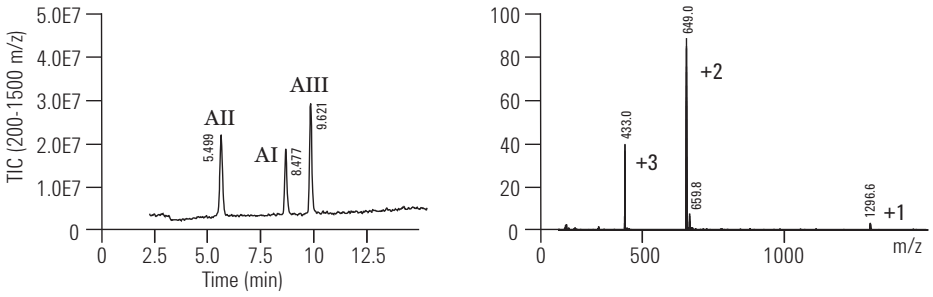
Flow Rate: 0.2 mL/min
Gradient: 15-50% B in 15 min
Temperature: 35 °C

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

A
Angiotensin I
Max: 10889
Low pH



B
Angiotensin I
Max: 367225
High pH



LC30003

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

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

Long life at high pH with 300Extend-C18

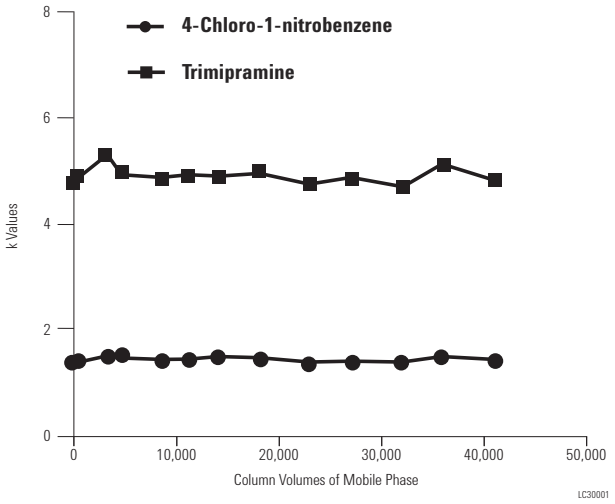
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

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

Flow Rate: 1.5 mL/min

Temperature: Aging 24 °C
Tests 40 °C

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



Use ZORBAX Extend-C18 for alternate selectivity at high pH

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

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

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

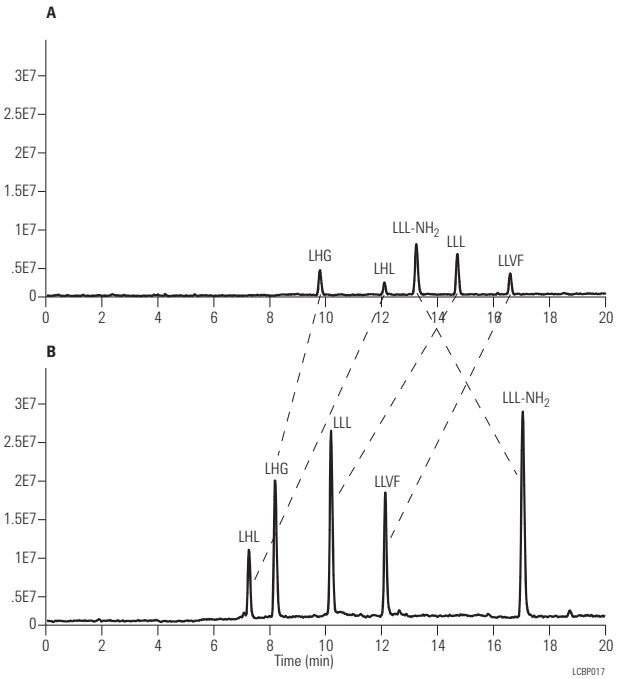
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

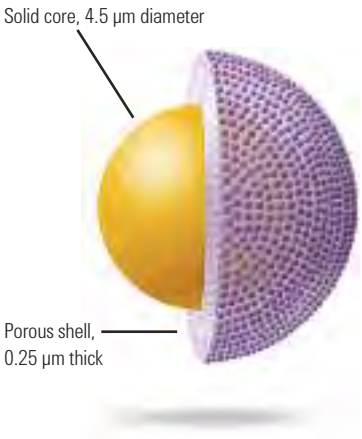
MS Conditions: Pos. Ion ESI-Vf 70V, Vcap 4.5 kV
N_2 – 35 psi, 12 L/min, 300 °C
4 µL (50 ng each peptide)

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



ZORBAX 300Å Extend-C18

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Analytical	4.6 x 250	5	770995-902
	Analytical	4.6 x 150	5	773995-902
	Rapid Resolution	4.6 x 150	3.5	763973-902
	Rapid Resolution	4.6 x 100	3.5	761973-902
	Rapid Resolution	4.6 x 50	3.5	765973-902
	Narrow Bore RR	2.1 x 150	3.5	763750-902
	Narrow Bore RR	2.1 x 100	3.5	761775-902
	Narrow Bore RR	2.1 x 50	3.5	765750-902
ZGC	Guard Cartridge, 4/pk	4.6 x 12.5	5	820950-932
ZGC	Guard Cartridge, 4/pk	2.1 x 12.5	5	821125-932
ZGC	Guard Hardware Kit			820999-901
Capillary Glass-lined Columns				
	Capillary RR	0.3 x 150	3.5	5065-4464
	Capillary RR	0.3 x 100	3.5	5065-4465
	Capillary RR	0.3 x 75	3.5	5065-4466
	Capillary RR	0.3 x 50	3.5	5065-4467



Poroshell 300

- UHPLC separations of biomolecules with superficially porous particles
- 300Å pore provide high efficiency and recovery with proteins (up to 1,000 kDa) and monoclonal antibodies
- Achieve long lifetime at low pH with Poroshell 300SB; at high pH with 300Extend-C18
- Optimize recovery and selectivity with four different bonded phases – 300SB-C18, 300SB-C8, 300SB-C3, and 300Extend-C18

Agilent Poroshell 300 columns are ideal for fast separations of proteins and peptides because the superficially porous particle allows for fast flow rates to be used while maintaining sharp, efficient peaks. Peptides and proteins are typically separated slowly to reduce the potential peak broadening of these slow diffusing analytes. However, Poroshell columns use a superficially porous particle made with a thin layer of porous silica, 0.25 µm thick, on a solid core of silica. This reduces the diffusion distance for proteins making practical rapid HPLC separations of peptides and proteins up to 500-1,000 kDA possible with 400/600 bar HPLC systems, including the Agilent 1260 Infinity Bio-inert. Poroshell columns bonded with StableBond bonded phases provide excellent stability and selectivity choices with TFA and formic acid mobile phases. The Poroshell 300Extend-C18 column can be used from pH 2-11 for unique separations. These columns can be used for analytical protein separations as well as LC/MS separations.

Poroshell 300 Columns

Column Specifications

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

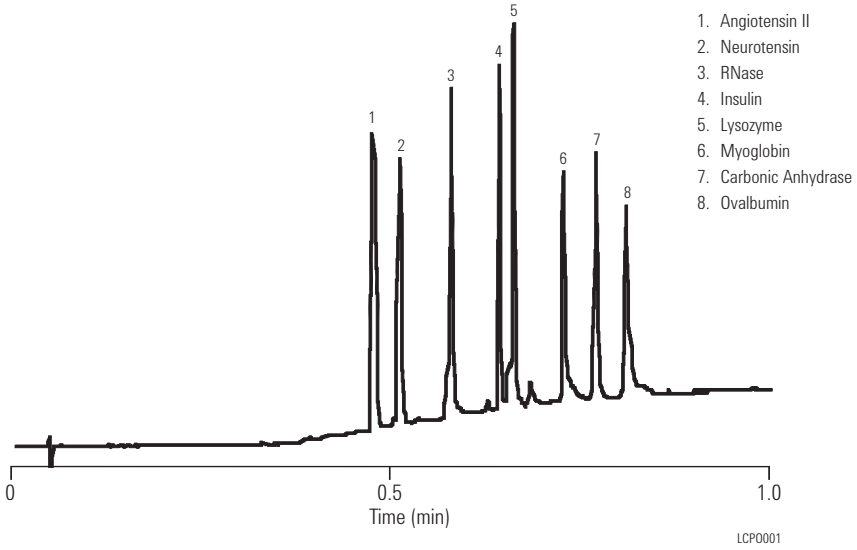
Specifications represent typical values only.

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

Poroshell 300 columns separate proteins and peptides in seconds

Column:	**Poroshell 300SB-C18 660750-902 2.1 x 75 mm, 5 µm**
Mobile Phase:	A: 0.1% TFA in H_20 B: 0.07% TFA in ACN
Flow Rate:	3.0 mL/min
Gradient:	5-100% B in 1.0 min
Temperature:	70 °C, 260 bar pressure
Detector:	215 nm
Sample:	Proteins and Peptides

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



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

TIPS & TOOLS

Further information can be found in the following publications:

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

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

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

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

www.agilent.com/chem/library

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

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

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

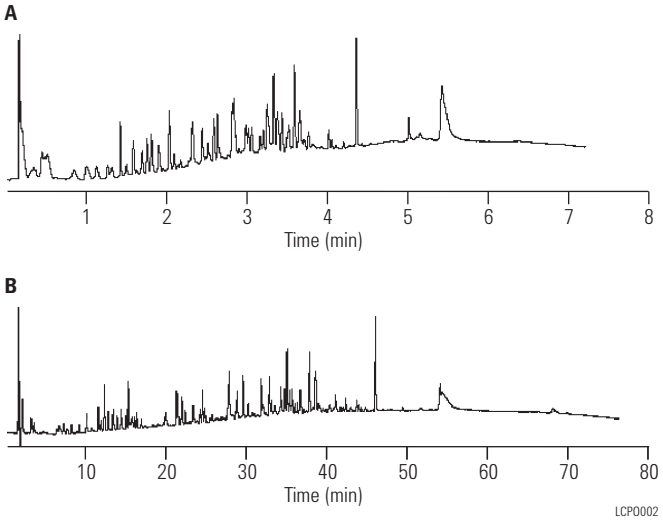
Mobile Phase: A: 95% H_2O, 5% ACN, 0.1% TFA
B: 5% H_2O, 95% ACN, 0.07% TFA

Flow Rate: 1 mL/min
0.208 mL/min

Gradient: 0-100% B = 12 min
0-100% B = 120 min

Temperature: 70 °C

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



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

MicroBore Poroshell 300 columns provide maximum sensitivity for LC/MS

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

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

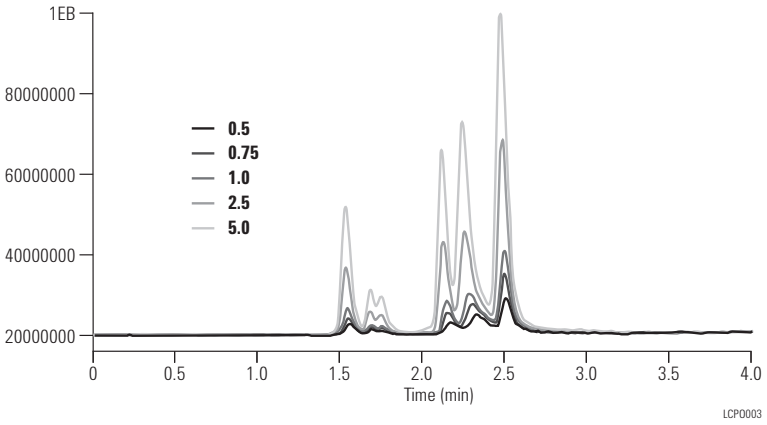
Flow Rate: 600 µL/min

Gradient: 20-100% B in 5.5 min

Temperature: 80 °C

MS Conditions: LC/MS: Pos. Ion ESI – Vcap 6000 V
Drying Gas Flow: 12 L/min
Drying Gas Temperature: 350 °C
Nebulizer: 45 psi
Fragmentor Volatage: 140 V
Scan: 600-2500
Stepsize: 0.15 amu
Peak width: 0.06 min

Sample: 1 µL



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

Monoclonal IgG1 chains: Separation on Poroshell 300SB-C8

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

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

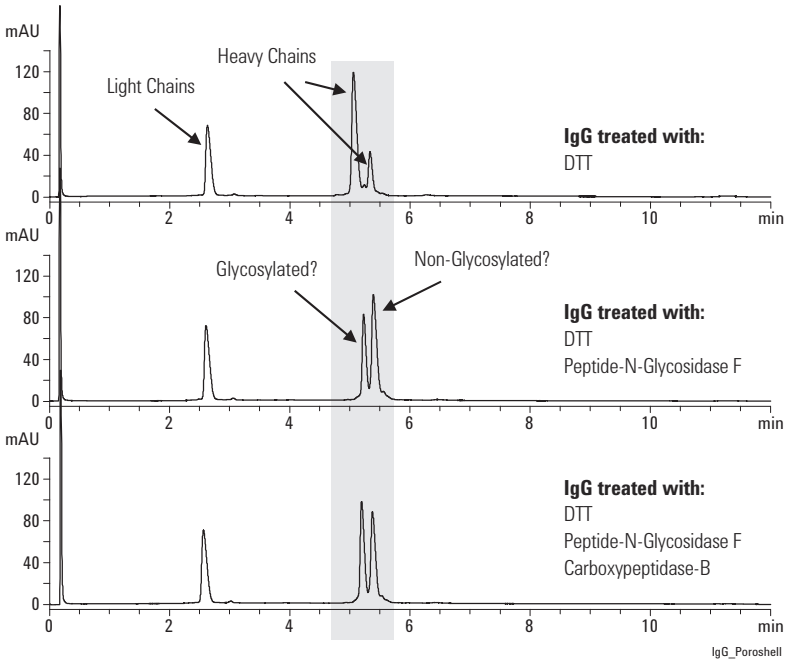
Flow Rate: 1.0 mL/min

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

Temperature: 70 °C

Sample: Monoclonal IgG1

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



TIPS & TOOLS

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

www.agilent.com/chem/library

Protein elution pattern on ZORBAX Poroshell 300SB-C8

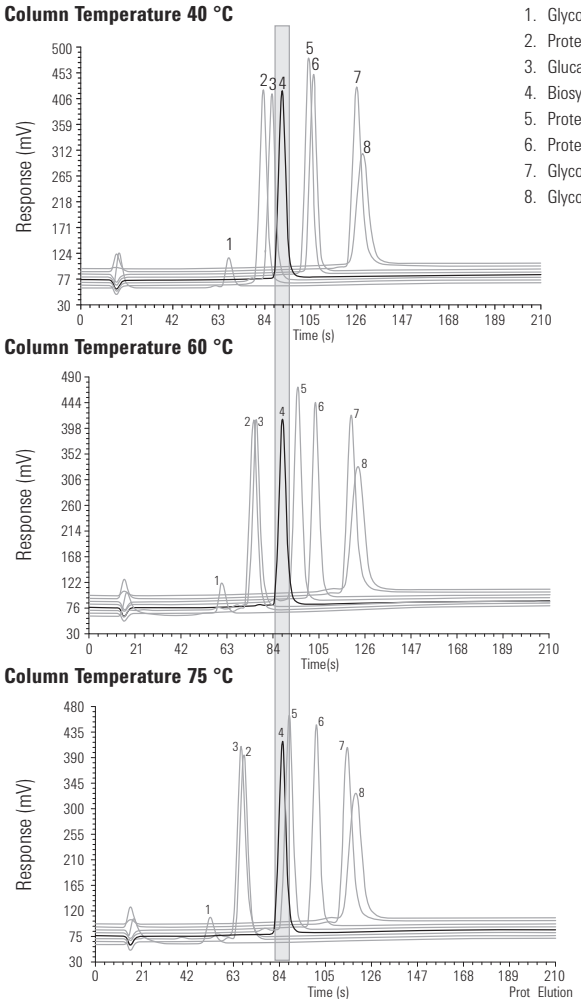
Column: **Poroshell 300SB-C8 660750-906 2.1 x 75 mm, 5 µm**

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

Flow Rate: 1.0 mL/min

Gradient: B: 20 to 70% in 3 min

Detector: UV (214 nm)



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

Poroshell 300

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

Poroshell 120

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

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

Column Specifications

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

Specifications represent typical values only

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

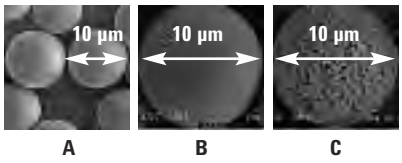
Poroshell 120

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

PLRP-S

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

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



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

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

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

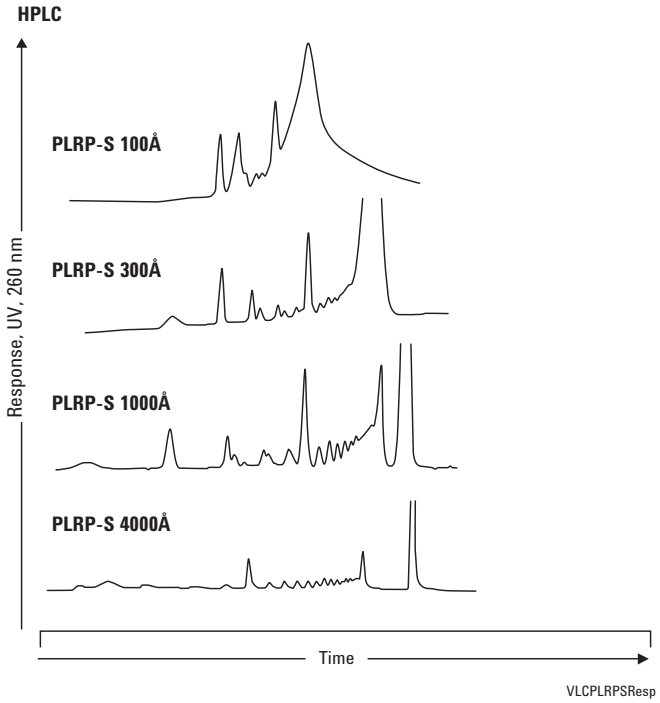
HPLC of 25 bp DNA ladder

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

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

Flow Rate: 200 µL/min

Gradient: 12.5-50% B in 150 min



Polyethylene glycols

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

Mobile Phase: A: Water
B: ACN

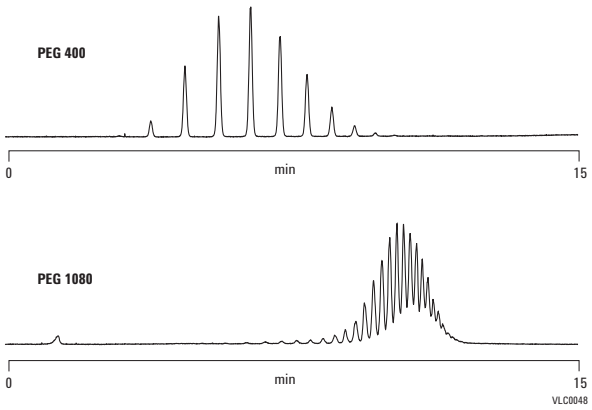
Gradient: 10-30% B in 12 min, held at 30% B for 3 min

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

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



Exploiting chemical stability – TFA concentration

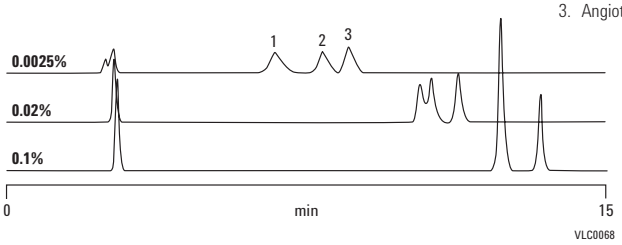
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

Gradient: Linear 12-40% B in 15 min

Flow Rate: 1.0 mL/min

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



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

Selectivity in peptide RP-LC

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

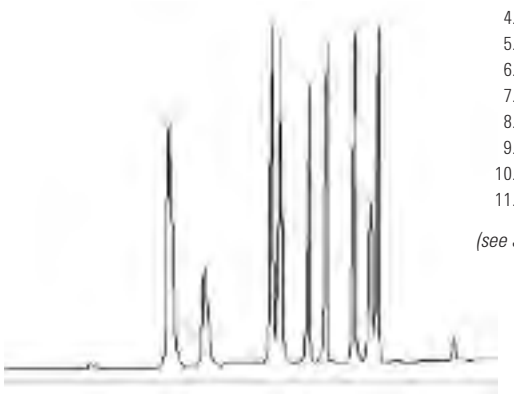
Mobile Phase: A: 0.1% TFA/1% 2-Propanol/Water
B: 0.1% TFA/1% 2-Propanol/ACN

Flow Rate: 1.0 mL/min

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

Detector: UV, 220 nm

Good separation of peptide standards on Agilent PLRP-S



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

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

Exploiting chemical stability – NH_4OH concentration

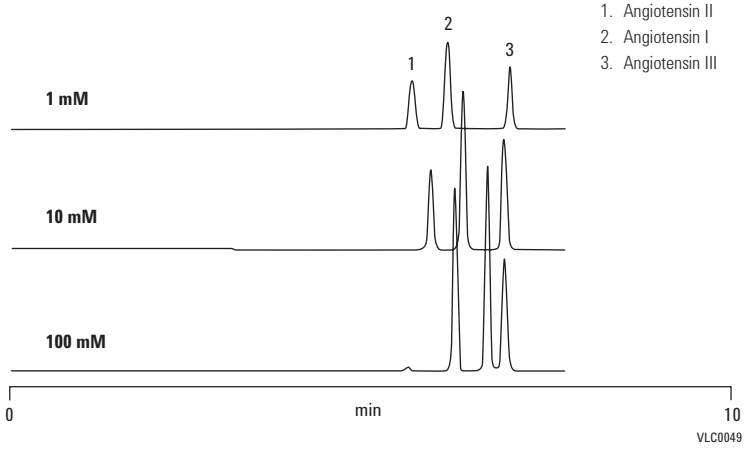
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

Gradient: Linear 10-100% B in 15 min

Flow Rate: 1.0 mL/min

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



Alberta Peptide Institute test mix

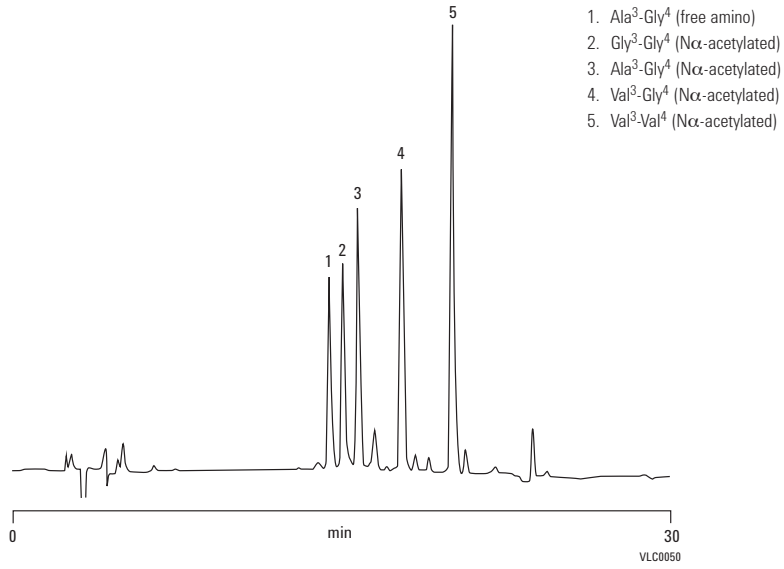
Column: **PLRP-S 100Å**
PL1512-5500
4.6 x 250 mm, 5 µm

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

Gradient: 0-100% B in 30 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



Whey proteins in dairy samples – milk

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

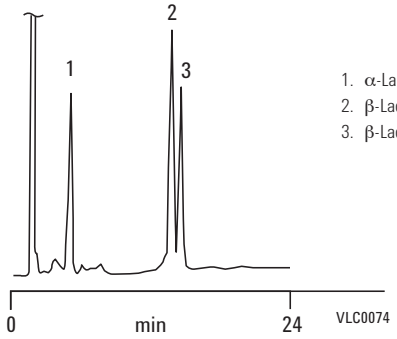
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

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

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Detector: UV, 220 nm



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

Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion-pair reversed-phase HPLC

Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

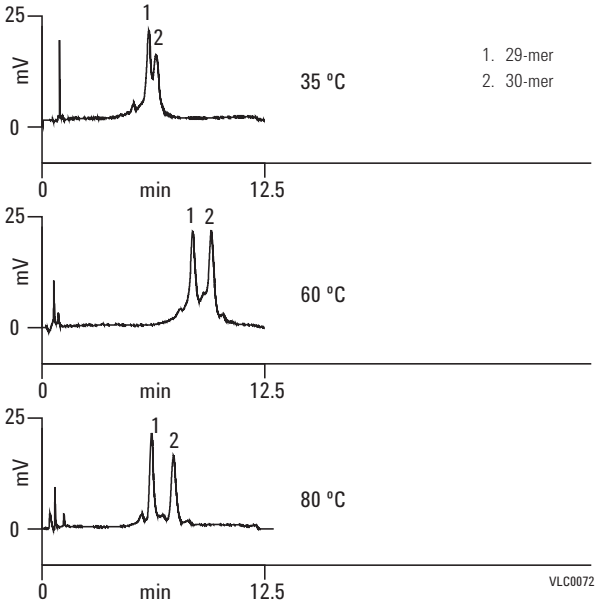
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

Gradient: 5% change in buffer B over 5 min

Flow Rate: 1.0 mL/min

Temperature: 35 °C, 60 °C, or 80 °C

Detector: UV, 254 nm



1. 29-mer
2. 30-mer

Large fibrous proteins

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

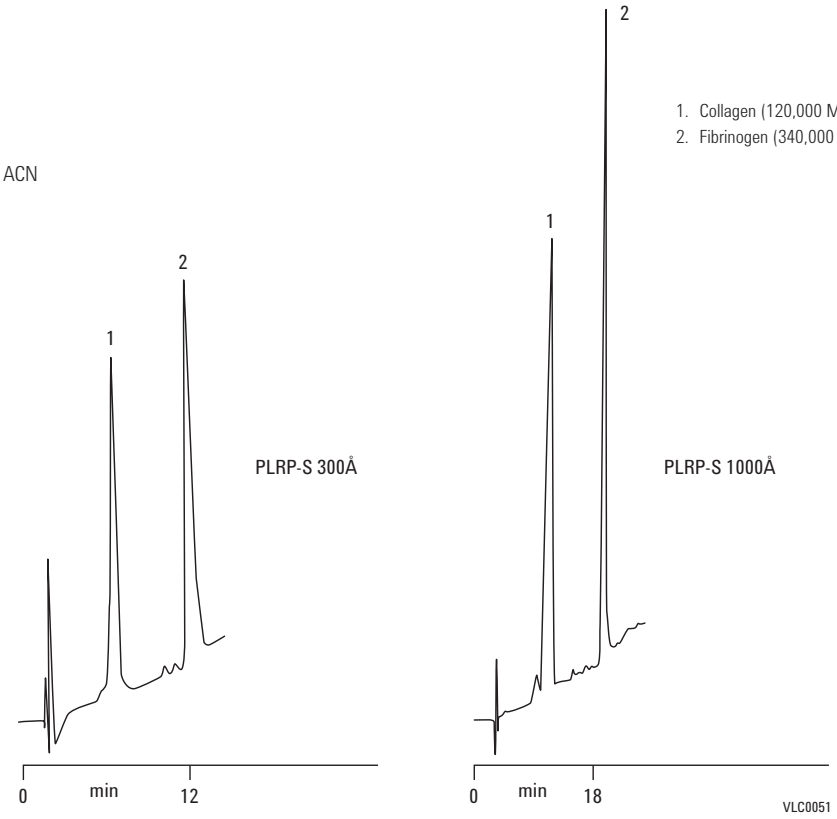
Column: **PLRP-S 1000Å**
PL1512-3802
4.6 x 150 mm, 8 µm

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

Flow Rate: 1.0 mL/min

Gradient: 20-60% B in 15 min

Detector: UV, 220 nm



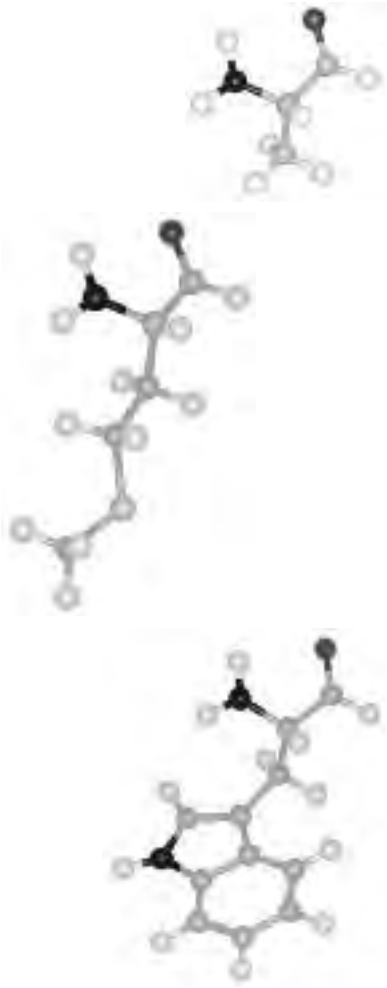
PLRP-S HPLC Columns

Hardware	Size (mm)	Particle Size (µm)	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
	4.6 x 250	8	PL1512-5800	PL1512-5801	PL1512-5802	
	4.6 x 150	8	PL1512-3800	PL1512-3801	PL1512-3802	PL1512-3803
	4.6 x 50	8		PL1512-1801	PL1512-1802	PL1512-1803
	4.6 x 250	5	PL1512-5500	PL1512-5501		
	4.6 x 150	5	PL1111-3500	PL1512-3501		
	4.6 x 50	5	PL1512-1500	PL1512-1501	PL1512-1502	PL1512-1503
	4.6 x 150	3	PL1512-3300	PL1512-3301		
	4.6 x 50	3	PL1512-1300	PL1512-1301		
	2.1 x 250	8		PL1912-5801		
	2.1 x 150	8		PL1912-3801	PL1912-3802	PL1912-3803
	2.1 x 50	8		PL1912-1801	PL1912-1802	PL1912-1803
	2.1 x 250	5	PL1912-5500	PL1912-5501		
	2.1 x 150	5	PL1912-3500	PL1912-3501		
	2.1 x 50	5	PL1912-1500	PL1912-1501	PL1912-1502	PL1912-1503
	2.1 x 150	3	PL1912-3300	PL1912-3301		
	2.1 x 50	3	PL1912-1300	PL1912-1301		
	1.0 x 50	8			PL1312-1802	
	1.0 x 50	5	PL1312-1500		PL1312-1502	
	1.0 x 10	5			PL1C12-2502	
	1.0 x 150	3	PL1312-3300			
	1.0 x 50	3	PL1312-1300			
PL	PLRP-S Guard Cartridges for 5 x 3 mm, 2/pk		PL1612-1801	PL1612-1801	PL1612-1801	PL1612-1801
PL	Guard Cartridge holder for 3.0 x 5.0 mm cartridges		PL1310-0016	PL1310-0016	PL1310-0016	PL1310-0016

TIPS & TOOLS

For prep columns and media ordering information, turn to pages 470-471.

For microbore columns ordering information, turn to page 463.



Amino Acid Analysis (AAA) Columns and Supplies

ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

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

The Agilent ZORBAX Eclipse AAA high efficiency column rapidly separates amino acids following an updated and improved protocol. Total analysis from injection-to-injection can be achieved in as little as 8 min (7 min analysis time) on a 50 mm 1.8 µm column, 14 min (9 min analysis time) on shorter, 75 mm length columns and 24 min (18 min analysis time) on the 150 mm column length. Exceptional sensitivity (5-50 pmol with DAD, FLD) and reliability are achieved using both OPA and FMOC derivatization chemistries in one fully automated procedure using the Agilent 1100/1200 HPLC instrument.

ZORBAX Eclipse Plus C18 columns are another excellent choice for Amino Acid Analysis. For more information about ZORBAX Eclipse Plus Columns, see page 248.

ZORBAX Eclipse Amino Acid Analysis (AAA) Columns

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	Analytical routine sensitivity	4.6 x 150	5	993400-902
	Analytical routine sensitivity, high-resolution using FLD	4.6 x 150	3.5	963400-902
	Analytical routine sensitivity, high-throughput	4.6 x 75	3.5	966400-902
	Solvent Saver high sensitivity, high-resolution	3.0 x 150	3.5	961400-302
ZGC	Guard Cartridges, 4/pk	4.6 x 12.5	5	820950-931
ZGC	Guard Hardware Kit			820999-901

TIPS & TOOLS

Further information can be found in the following publication:

High-Speed Amino Acid Analysis (AAA) on 1.8 µm Reversed-Phase (RP) Columns (publication # 5989-6297EN)

www.agilent.com/chem/library

Amino Acid Standards

Each amino acid standard contains the following amino acids:

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

Amino Acid Standards, 10 x 1 mL ampoules*

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

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

Amino Acid Separations Reagents

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

High resolution of 24 amino acids using ZORBAX Eclipse-AAA protocol

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

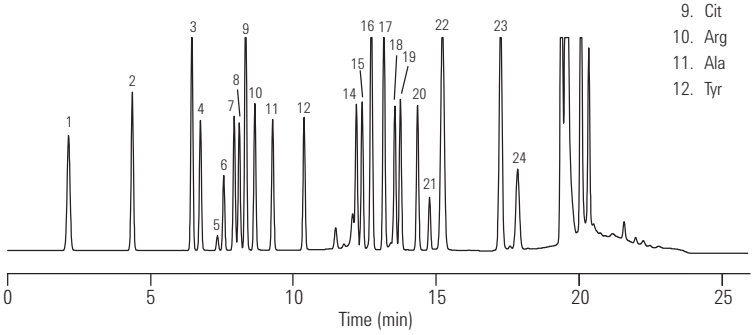
Mobile Phase: A: 40 mM Na_2HPO_4, pH 7.8
B: ACN:MeOH:Water, 45:45:10 v/v

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: Fluorescence

Sample: 24 Amino Acids



LCPAH01

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

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

Ion-Exchange Chromatography

Purify proteins and other charged molecules

Ion-exchange chromatography (IEX) is a highly sensitive technique that allows you to separate ions and polar molecules based on their charge. Like SEC, IEX can be used to separate proteins in their native state.

Applying IEX to charge variant analysis

During production and purification, antibodies can exhibit changes in charge heterogeneity as a result of amino acid substitutions, glycosylation, phosphorylation, and other post-translational or chemical modifications. Because these changes can impact stability and activity – or cause immunologically adverse reactions – the analysis of charge heterogeneity in monoclonal antibody (MAb) preparations is critical to biopharmaceuticals.

In protein analysis, charge variations at a given pH indicate a change in the primary molecular structure – resulting in additional forms of the protein in question. These are called isoforms (or charge variants), and can be resolved by IEX chromatography. IEX is also useful as a preparative technique.

The pages that follow describe Agilent's family of weak and strong ion-exchangers – both anionic and cationic.

- **Agilent non-porous Bio IEX columns** are designed for high-resolution, high-efficiency, and high-recovery separations.
- **Agilent Bio MAb columns** are optimized for separating charge isoforms of monoclonal antibodies.
- **Agilent porous IEX columns (PL-SAX and PL-SCX)** are chemically stable, and are available in two pore sizes – allowing you to separate peptides, oligonucleotides, and very large proteins.
- **Bio-Monolith IEX columns** are uniquely suited to separating antibodies, viruses, and DNA.

Ion-Exchange Column Selection

Application	Agilent Columns	Notes
Monoclonal antibodies	Agilent Bio MAb	Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution charge-based separations of monoclonal antibodies.
Peptides and proteins	Agilent Bio IEX	Agilent Bio Ion-Exchange columns are packed with polymeric, nonporous, ion-exchange particles. Bio IEX columns are designed for high resolution, high recovery and highly efficient separations.
Proteins, peptides and deprotected synthetic oligonucleotides	PL-SAX • 1000Å • 4000Å	The strong anion-exchange functionality, covalently linked to a fully porous chemically stable polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. The 5 µm media delivers separations at high resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins and peptides	PL-SAX 1000Å	
Very large biomolecules/high speed	PL-SAX 4000Å	
Small peptides to large proteins	PL-SCX • 1000Å • 4000Å	PL-SCX is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation and purification of a wide range of biomolecules. The 5 µm media delivers separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.
Globular proteins	PL-SCX 1000Å	
Very large biomolecules/high speed	PL-SCX 4000Å	
Antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro biomolecules	Bio-Monolith • Bio-Monolith QA • Bio-Monolith DEAE • Bio-Monolith SO_3 • Bio-Monolith Protein A	Strong cation-exchange, strong and weak anion-exchange, and Protein A phases. Bio-Monolith HPLC columns are compatible with preparative LC systems, including Agilent 1100 and 1200 HPLC systems.
Viruses, DNA, large proteins	Bio-Monolith QA	
Plasmid DNS, bacteriophages	Bio-Monolith DEAE	
Proteins, antibodies	Bio-Monolith SO_3	

Agilent Bio MAb HPLC Columns

- A packing support composed of a rigid, spherical, highly cross-linked polystyrene divinylbenzene (PS/DVB) non-porous bead
- Particles grafted with a hydrophilic, polymeric layer, virtually eliminating non-specific binding of antibody proteins
- A different process is used to layer the weak cation-exchange phase to the particle making it a higher density than the Agilent Bio WCX column particles
- Specifically designed for the separation of charge isoforms of monoclonal antibodies

Thorough characterization of monoclonal antibodies includes the identification and monitoring of acidic and basic isoforms. Agilent Bio MAb HPLC columns feature a unique resin specifically designed for high-resolution, charge-based separations of monoclonal antibodies. Compatible with aqueous solution buffers, acetonitrile/acetone/methanol and water mixtures. Commonly used buffers: phosphate, tris, MES and acetate.

Bio MAb columns are available in 1.7, 3, 5 and 10 µm sizes, providing higher resolution with smaller particles.

Column Specifications

Bonded Phase	ID	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
Weak Cation-Exchange (carboxylate)	2.1 and 4.6 mm	1.7, 3, 5 and 10 µm	2-12	80 °C	0.1-1.0 mL/min

TIPS & TOOLS

Capillary electrophoresis is an alternative technique to liquid chromatography for the separation of charged isoforms. Further information can be found in the following Technical Note:

Capillary electrophoreseis focusing on the Agilent Capillary Electrophoresis system (publication # 5989-9852EN)

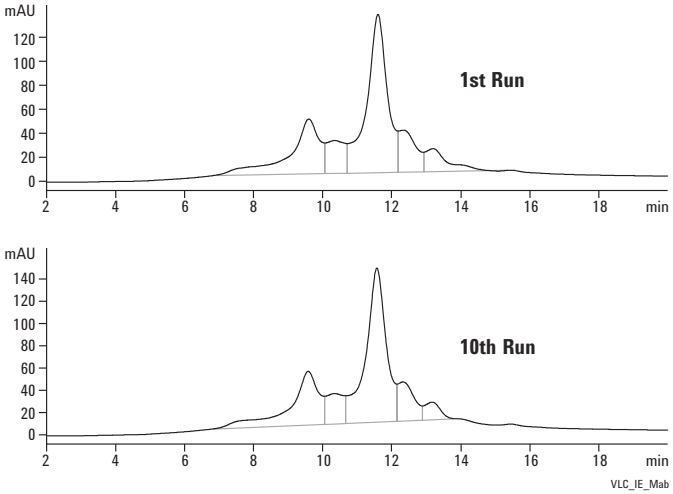
www.agilent.com/chem/library

Consistent ion-exchange MAb separation

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

Buffer:	A: Sodium phosphate buffer, 20 mM B: Buffer A + 400 mM NaCl
Gradient:	15-35% Buffer B from 0-30 min
Flow Rate:	0.65 mL/min
Sample:	CHO-humanized MAb, 1 mg/mL
Injection:	2.5 µL
Detector:	UV 220 nm
Temperature:	Ambient

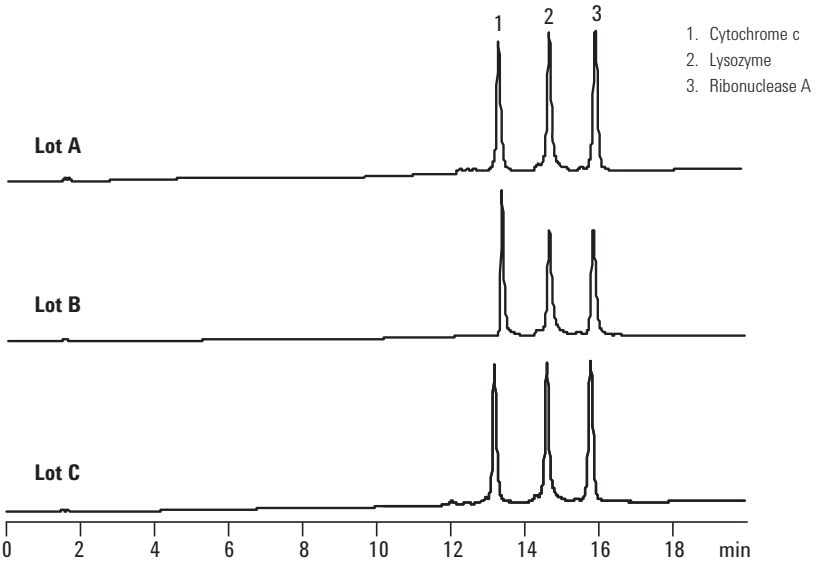
To provide a metal free flow path, Bio MAb PEEK columns are available.



Virtually eliminate retention time variations

Column: **Bio MAb, stainless steel**
5190-2413
4.6 x 250 mm, 10 µm

Mobile Phase:	A: 10 mM phosphate, pH 6.0 B: A + 1.0 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	0-100% B in 42 min
Temperature:	25 °C
Detector:	UV 214 nm



The combination of well-controlled resin production, column surface chemistry, and column packing virtually eliminates retention time variations from column-to-column and lot-to-lot.

Charge isoform analysis of monoclonal antibodies

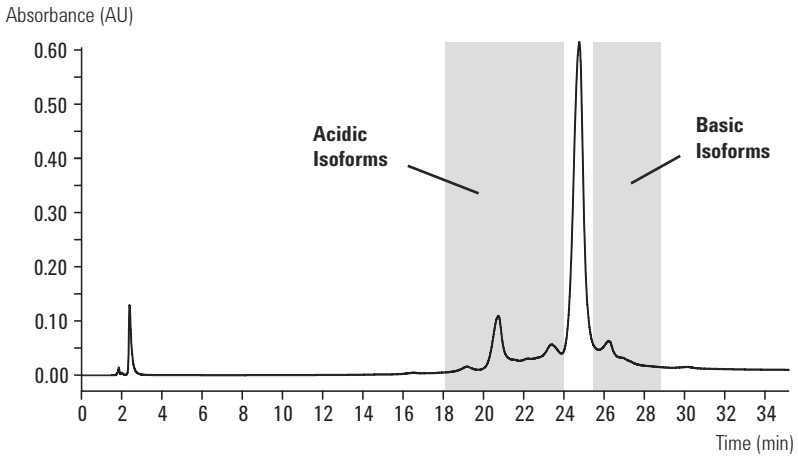
Column: **Bio MAb, PEEK 5190-2407 4.6 x 250 mm, 5 µm**

Mobile Phase: A: 10 mM Sodium Phosphate, pH 7.50
B: A + 100 mM NaCl, pH 7.50

Flow Rate: 0.8 mL/min

Gradient: 15-95% B in 60 min

Sample: 5 µL, 5 mg/mL, MAb



High resolution separation of acidic and basic charge variants using the Agilent Bio MAb NP5 column

Agilent Bio MAb HPLC Columns

Size (mm)	Particle Size (µm)	Bio MAb PEEK	Pressure Limit	Bio MAb Stainless Steel	Pressure Limit
4.6 x 250	10	5190-2415	275 bar, 4000 psi	5190-2413	275 bar, 4000 psi
4.6 x 50, Guard	10	5190-2416	275 bar, 4000 psi		
4.6 x 250	5	5190-2407	400 bar, 5800 psi	5190-2405	413 bar, 6000 psi
4.6 x 50, Guard	5	5190-2408	400 bar, 5800 psi		
4.6 x 50	3			5190-2403	551 bar, 8000 psi
4.6 x 50	1.7			5190-2401	600 bar, 8700 psi
4.0 x 10, Guard	10			5190-2414	275 bar, 4000 psi
4.0 x 10, Guard	5			5190-2406	413 bar, 6000 psi
4.0 x 10, Guard	3			5190-2404	551 bar, 8000 psi
4.0 x 10, Guard	1.7			5190-2402	600 bar, 8700 psi
2.1 x 250	10	5190-2419	275 bar, 4000 psi		
2.1 x 50, Guard	10	5190-2420	275 bar, 4000 psi		
2.1 x 250	5	5190-2411	400 bar, 5800 psi		
2.1 x 50, Guard	5	5190-2412	400 bar, 5800 psi		

Agilent Bio IEX HPLC Columns

- Highly cross-linked and rigid nonporous poly(styrene divinylbenzene) (PS/DVB) particles are grafted with a hydrophilic, polymeric layer, eliminating nonspecific binding
- Uniform, densely packed ion-exchange functional groups are chemically bonded to the hydrophilic layer (multiple ion-exchange groups per anchoring) to increase column capacity
- Particles, coating and bonding are resistant to high pressures, promoting higher resolution and faster separations
- Multiple ion-exchange groups are captured on one anchoring to increase capacity

Agilent Bio IEX HPLC columns are packed with polymeric, nonporous, ion-exchange particles and are designed for high resolution, high recovery and highly efficient separations of peptides, oligonucleotides and proteins.

The Bio IEX family offers strong cation-exchange (SCX), weak cation-exchange (WCX), strong anion-exchange (SAX) and weak anion-exchange (WAX) phases. All phases are available in 1.7, 3, 5 and 10 µm non-porous particles sizes.

Column Specifications

Bonded Phase	ID	Particle Size	pH Stability	Operating Temperature Limit	Flow Rate
SCX (Strong cation-exchange) - SO_3H	2.1 and 4.6 mm	1.7, 3, 5 and 10 µm	2-12	80 °C	0.1-1.0 mL/min
WCX (Weak cation-exchange) - COOH					
SAX (Strong anion-exchange) - $N(CH_3)_3$					
WAX (Weak cation-exchange) - $N(C_2H_5)_2$					

TIPS & TOOLS

More information is a click away. We have a variety of educational primers, application notes, maintenance guides, and literature available from Agilent for free.

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

Exceptional separating power

Column: **Agilent Bio SCX, stainless steel**
5190-2423
4.6 x 50 mm, 3 µm

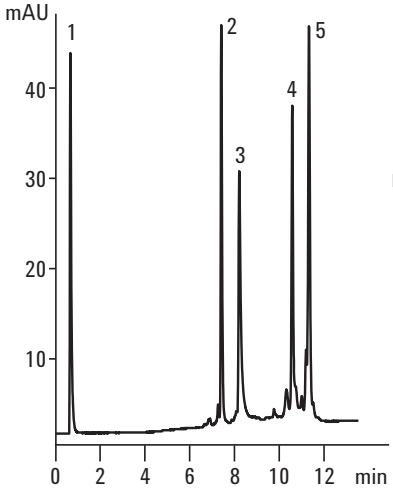
Buffer: 10 mM Phosphate, pH 6.0

Flow Rate: 0.5 mL/min

Gradient: 0-1.0 M NaCl, 15 min

Detector: 280 nm

The hydrophilic, polymeric layer and densely packed ion-exchange functional groups provide extremely sharp peak shapes and high resolution of a mixture of proteins with a broad range of isoelectric points (pI).



1. Ovalbumin, 4.6 pI
2. Ribonuclease A, 8.7 pI
3. Cytochrome c, 9.6 pI
4. Aprotinin, 10.0 pI
5. Lysozyme, 11.0 pI

N > 100,000/50 mm for Lysozyme

Separation of protein standards on Agilent 3 µm ion-exchange columns by cation-exchange chromatography

Column A: **Agilent Bio SCX, NP 3, 4.6 x 50 mm, SS**

Column B: **Agilent Bio WCX, NP 3, 4.6 x 50 mm, SS**

Column C: **Agilent Bio MAb, NP 3, 4.6 x 50 mm, SS**

Mobile Phase: A: 10 mM $NaH_2PO4.2H_2O$, pH 5.70
B: A + 1 M NaCl

Flow Rate: 0.5 mL/min

Gradient: 0 min - 100% A : 0% B
25 min - 0% A : 100% B

Temperature: Ambient

Detector: Agilent 1260 Infinity Bio-inert Quaternary LC with diode array detector at 220 nm

Sample: Cytochrome c, ribonuclease A, lysozyme and protein mix

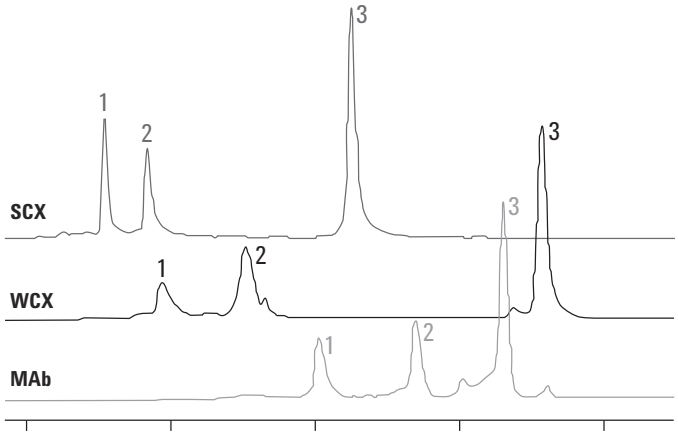


Illustration that Bio WCX, SCX and MAb columns are capable of producing protein separations

Agilent column	Peak number	Peak name	RT [min]	Height [mAU]	Area [mAU*s]	Plates	Width [min]	Resolution
Bio WCX NP, 3 µm	1	Cytochrome c	7.86	124	1833	7844	0.2089	-
	2	RNase A	9.03	241	3358	10800	0.2044	3.32
	3	Lysozyme	13.13	636	7274	44488	0.1466	13.73
Bio SCX NP, 3 µm	1	RNase A	7.06	396	2616	39847	0.0832	-
	2	Cytochrome c	7.66	297	2778	28920	0.1060	1.08
	3	Lysozyme	10.49	763	7186	44828	0.1167	1.37
Bio MAb NP, 3 µm	1	Cytochrome c	10.04	203	2369	21814	0.1600	-
	2	RNase A	11.37	256	2690	33314	0.1467	3.11
	3	Lysozyme	12.59	652	6616	56734	0.1244	5.28

Weak cation-exchange chromatography for P128 therapeutic protein sample on the Agilent 1260 Bio-inert Quaternary LC system using different cation-exchange columns

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

Column B: **Bio MAb, PEEK**
5190-2415
4.6 x 250 mm, 10 µm

Column C: **Brand B WCX-10**
4.0 x 250 mm, 10 µm

Mobile Phase: A: 20 mM sodium phosphate (pH = 6.0)
B:20 mM sodium phosphate (pH = 6.0) containing 1.0 M sodium chloride

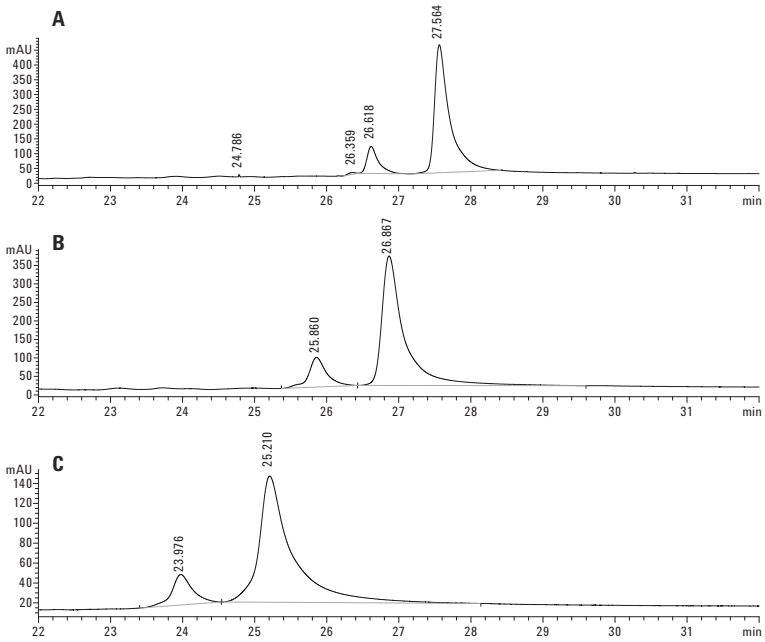
Flow Rate: 0.5 mL/min

Gradient: 10% B 0 min, 35% B 35 min, 10% B 36 min, 10% B 45 min

Detector: UV, 220 nm/4 nm, Reference: Off (data also acquired at 220, 230, 240, and 280 nm)

Sample: P128

Sample was desalted by ultrafiltration and extracted into 20 mM sodium phosphate.



Agilent Bio IEX HPLC Columns, PEEK

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
4.6 x 250	10	275 bar, 4000 psi	5190-2435	5190-2455	5190-2475	5190-2495
4.6 x 50, Guard	10	275 bar, 4000 psi	5190-2436	5190-2456	5190-2476	5190-2496
4.6 x 250	5	400 bar, 5800 psi	5190-2427	5190-2447	5190-2467	5190-2487
4.6 x 50, Guard	5	400 bar, 5800 psi	5190-2428	5190-2448	5190-2468	5190-2488
2.1 x 250	10	275 bar, 4000 psi	5190-2439	5190-2459	5190-2479	5190-2499
2.1 x 50, Guard	10	275 bar, 4000 psi	5190-2440	5190-2460	5190-2480	5190-2500
2.1 x 250	5	400 bar, 5800 psi	5190-2431	5190-2451	5190-2471	5190-2491
2.1 x 50, Guard	5	400 bar, 5800 psi	5190-2432	5190-2452	5190-2472	5190-2492

Agilent Bio IEX HPLC Columns, Stainless Steel

Size (mm)	Particle Size (µm)	Pressure Limit	Bio SCX Part No.	Bio WCX Part No.	Bio SAX Part No.	Bio WAX Part No.
4.6 x 250	10	275 bar, 4000 psi	5190-2433	5190-2453	5190-2473	5190-2493
4.6 x 250	5	413 bar, 6000 psi	5190-2425	5190-2445	5190-2465	5190-2485
4.6 x 50	3	551 bar, 8000 psi	5190-2423	5190-2443	5190-2463	5190-2483
4.6 x 50	1.7	600 bar, 8700 psi	5190-2421	5190-2441	5190-2461	5190-2481
4.0 x 10, Guard	10	275 bar, 4000 psi	5190-2434	5190-2454	5190-2474	5190-2494
4.0 x 10, Guard	5	413 bar, 6000 psi	5190-2426	5190-2446	5190-2466	5190-2486
4.0 x 10, Guard	3	551 bar, 8000 psi	5190-2424	5190-2444	5190-2464	5190-2484
4.0 x 10, Guard	1.7	275 bar, 4000 psi	5190-2422	5190-2442	5190-2462	5190-2482

PL-SAX Strong Anion-Exchange Columns

- Small particles deliver excellent chromatographic performance
- Wide range of particle sizes and 2 pore sizes for flexible analysis to scale-up purification
- Exceptional stability for long column lifetime

PL-SAX -$N(CH_3)_3^+$ is ideal for the anion-exchange HPLC separations of proteins, peptides and deprotected synthetic oligonucleotides under denaturing conditions. The strong anion-exchange functionality, covalently linked to a chemically stable fully porous polymer, extends the operating pH range. In addition, the anion-exchange capacity is independent of pH. For synthetic oligonucleotides, separations using denaturing conditions of temperature, organic solvent, and high pH are all possible. PL-SAX delivers improved chromatography for self-complementary or G-rich sequences that may associate to form aggregates or hairpin structures. The 5 µm material provides high efficiency separations of n and n-1 sequences. A wide range of particle sizes and column geometries permits analysis scale-up to purification. The strong anion-exchange functionality provides a material with exceptional chemical and thermal stability, even with sodium hydroxide eluents, leading to long column lifetime.

Column Specifications

Bonded Phase	ID (mm)	Particle Size (µm)	Pore Size	pH Stability	Operating Temperature Limit
Strong Anion-Exchange	2.1, 4.6, 7.5, 25, 50 and 100	5, 8, 10 and 30	1000Å and 4000Å	1-14	80 °C

Standard ion-exchange protein separation

Column: **PL-SAX 1000Å**
PL1551-1502
4.6 x 50 mm, 5 µm

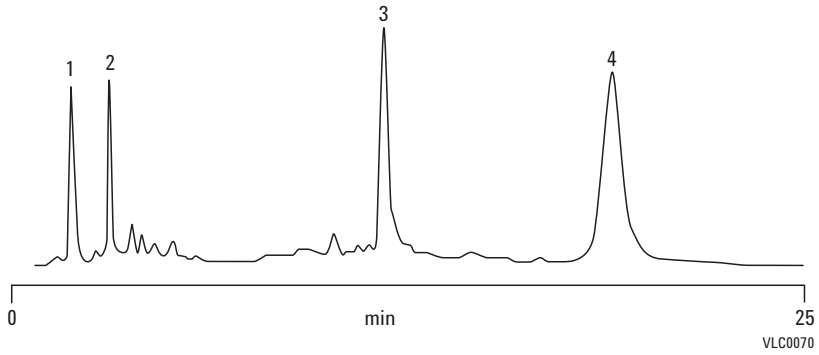
Mobile Phase: A: 10 mM Tris HCl pH 8
B: A+0.35 M NaCl pH 8

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

1. Myoglobin
2. Bovine carbonic anhydrase
3. Ovalbumin
4. Soybean trypsin inhibitor



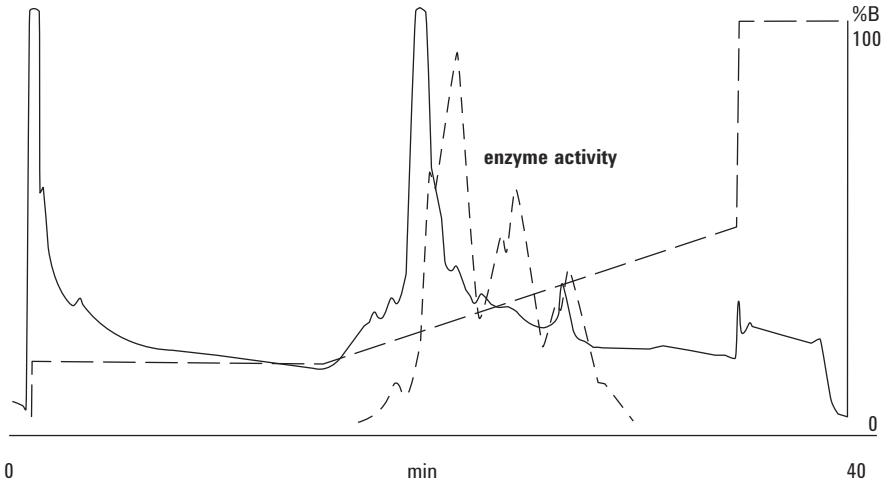
Analysis of choline kinase on PL-SAX 4000Å

Column: **PL-SAX**
PL1551-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 20 mM Tris 5% ethylene glycol, pH 7.5
(The following are required to retain enzyme activity)
1.0 mM Ethylene glycol tetraacetic acid
2.0 mM ß-Mercaptoethanol
0.2 mM Phenylmethylsulfonyl fluoride
B: A + 1 M KCl

Flow Rate: 3.0 mL/min

Detector: UV, 280 nm



Separation courtesy of T Porter, Purdue University, USA

Analysis of representative whey proteins

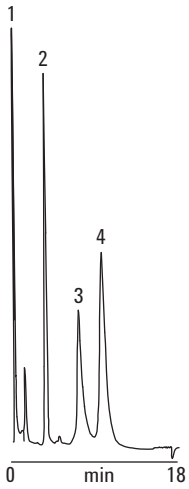
Column: **PL-SAX 1000Å**
PL1551-1802
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.02 M Tris HCl, pH 7
B: A + 0.5 M CH_3COONa, pH 7

Flow Rate: 1.0 mL/min

Gradient: Linear 0-50% B in 10 min

Detector: UV, 280 nm



1. Carbonic anhydrase
2. α-lactalbumin
3. β-lactoglobulin B
4. β-lactoglobulin A

High resolution separation of a Poly-T-Oligonucleotide size standard spiked with 10-mer, 15-mer, 30-mer and 50-mer (main peaks)

Column: **PL-SAX 1000Å**
PL1551-1802
4.6 x 50 mm, 8 µm

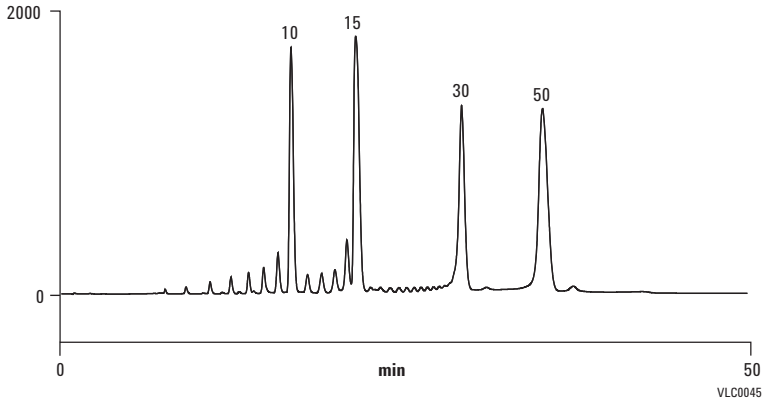
Mobile Phase: A: 7:93 v/v ACN: 0.1 M TEAA, pH 8.5
B: 7:93 v/v ACN: 0.1 M TEAA,
1 M ammonium chloride, pH 8.5

Gradient: 0-40% B in 10 min, followed by
40-70% B in 14 min and
70-100% B in 25 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 220 nm

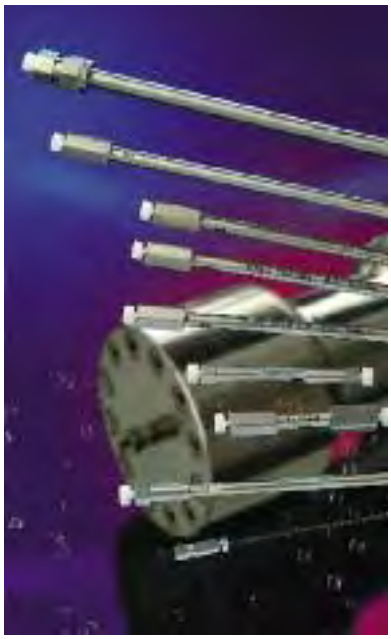


PL-SAX Strong Anion-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SAX 1000Å	PL-SAX 4000Å
1.0 x 50	5	207 bar, 3000 psi	PL1351-1502	PL1351-1503
2.1 x 50	5	207 bar, 3000 psi	PL1951-1502	PL1951-1503
4.6 x 50	5	207 bar, 3000 psi	PL1551-1502	PL1551-1503
2.1 x 50	8	207 bar, 3000 psi	PL1951-1802	PL1951-1803
2.1 x 150	8	207 bar, 3000 psi	PL1951-3802	PL1951-3803
4.6 x 50	8	207 bar, 3000 psi	PL1551-1802	PL1551-1803
4.6 x 150	8	207 bar, 3000 psi	PL1551-3802	PL1551-3803
4.6 x 250	10	207 bar, 3000 psi	PL1551-5102	PL1551-5103
4.6 x 150	10	207 bar, 3000 psi	PL1551-3102	PL1551-3103
25 x 50	10	207 bar, 3000 psi	PL1251-1102	PL1251-1103
25 x 150	10	207 bar, 3000 psi	PL1251-3102	PL1251-3103
50 x 150	10	207 bar, 3000 psi	PL1751-3102	PL1751-3103
100 x 300	10	207 bar, 3000 psi	PL1851-2102	PL1851-2103
4.6 x 250	30	207 bar, 3000 psi	PL1551-5702	PL1551-5703
4.6 x 150	30	207 bar, 3000 psi	PL1551-3702	PL1551-3703
25 x 150	30	207 bar, 3000 psi	PL1251-3702	PL1251-3703
50 x 150	30	207 bar, 3000 psi	PL1751-3702	PL1751-3703
100 x 300	30	207 bar, 3000 psi	PL1851-3102	PL1851-3103

PL-SAX Strong Anion-Exchange Bulk Media

Size	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å
100 g	10	PL1451-4102	PL1451-4103
1 kg	10	PL1451-6102	PL1451-6103
100 g	30	PL1451-4702	PL1451-4703
1 kg	30	PL1451-6702	PL1451-6703



PL-SCX Strong Cation-Exchange Columns

- Optimal design for effective separation of biomolecules
- Pore sizes allow use of a range of solute sizes
- Exceptional stability for long column lifetime

PL-SCX -SO_3^- is a macroporous PS/DVB matrix with a very hydrophilic coating and strong cation-exchange functionality. This process is controlled to provide the optimum density of strong cation-exchange moieties for the analysis, separation and purification of a wide range of biomolecules, from small peptides to large proteins. Two pore sizes are available, 1000Å and 4000Å, to provide good mass transfer characteristics for a range of solute sizes. The 5 µm media delivers separations at higher resolution with the 30 µm media used for medium pressure liquid chromatography.

Column Specifications

Bonded Phase	ID (mm)	Particle Size (µm)	Pore Size	pH Stability	Operating Temperature Limit
Strong Cation-Exchange	2.1, 4.6, 7.5, 25, 50 and 100	5, 8, 10 and 30	1000Å and 4000Å	1-14	80 °C

Standard protein separation

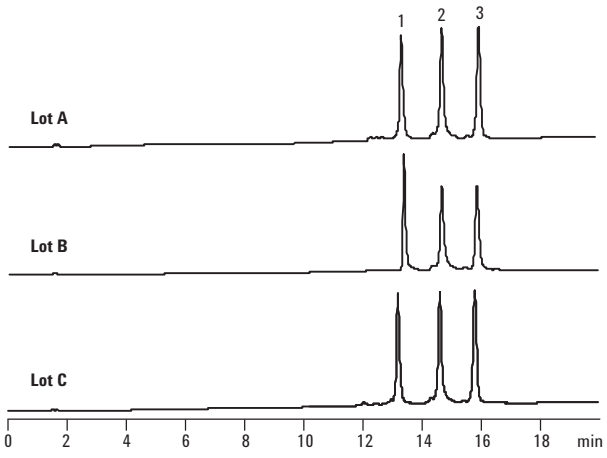
Column: **PL-SCX 1000Å**
PL1545-1502
4.6 x 50 mm, 5 µm

Mobile Phase: A: 20 mM KH_2PO_4, pH 6.0
B: A + 1 M NaCl

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Myoglobin
2. Chymotrypsinogen A
3. Cytochrome c
4. Lysozyme

PL-SCX Strong Cation-Exchange Columns

Size (mm)	Particle Size (µm)	Pressure Limit	PL-SCX 1000Å	PL-SCX 4000Å
1.0 x 50	5	207 bar, 3000 psi	PL1345-1502	PL1345-1503
2.1 x 50	5	207 bar, 3000 psi	PL1945-1502	PL1945-1503
4.6 x 50	5	207 bar, 3000 psi	PL1545-1502	PL1545-1503
2.1 x 50	8	207 bar, 3000 psi	PL1945-1802	PL1945-1803
2.1 x 150	8	207 bar, 3000 psi	PL1945-3802	PL1945-3803
4.6 x 50	8	207 bar, 3000 psi	PL1545-1802	PL1545-1803
4.6 x 150	8	207 bar, 3000 psi	PL1545-3802	PL1545-3803
4.6 x 150	10	207 bar, 3000 psi	PL1545-3102	PL1545-3103
4.6 x 250	10	207 bar, 3000 psi	PL1545-5102	PL1545-5103
25 x 50	10	207 bar, 3000 psi	PL1245-1103	PL1245-1103
25 x 150	10	207 bar, 3000 psi	PL1245-3103	PL1245-3103
50 x 150	10	207 bar, 3000 psi	PL1745-3103	PL1745-3103
100 x 300	10	207 bar, 3000 psi	PL1845-2103	PL1845-2103
4.6 x 150	30	207 bar, 3000 psi	PL1545-3702	PL1545-3703
4.6 x 250	30	207 bar, 3000 psi	PL1545-5703	PL1545-5703
25 x 150	30	207 bar, 3000 psi	PL1245-3702	PL1245-3703
50 x 150	30	207 bar, 3000 psi	PL1745-3703	PL1745-3703
100 x 300	30	207 bar, 3000 psi	PL1845-3102	PL1845-3103

PL-SCX Strong Cation-Exchange Bulk Media

Size	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å
100 g	10	PL1445-4102	PL1445-4102
1 kg	10	PL1445-6102	PL1445-6103
100 g	30	PL1445-4702	PL1445-4703
1 kg	30	PL1445-6702	PL1445-6703

Bio-Monolith Ion-Exchange HPLC Column

Agilent Bio-Monolith Ion-Exchange HPLC Columns

- Polymer-based, monolith HPLC columns designed for macro biomolecule separations
- Flow-rate independent separations; no diffusion, no pores and no void volume make transport between mobile and stationary phase very rapid
- Monolith disk is 5.2 mm x 4.95 mm (100 µL column volume) with continuous channels, eliminating diffusion mass transfer
- Extremely fast separations speed up method development time and decrease costs; locking in method parameters takes significantly less time and buffer

Agilent Bio-Monolith Ion-Exchange HPLC columns provide high resolution and rapid separations of antibodies (IgG, IgM), plasmid DNA, viruses, phages and other macro biomolecules. The product family offers strong cation-exchange, strong and weak anion-exchange and Protein A phases. Bio-Monolith HPLC columns are compatible with HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems.

Agilent Bio-Monolith HPLC Column Selection Guide

Column	Description	Key Applications	Part No.
Bio-Monolith QA	The quaternary amine bonded phase (Strong Anion-Exchange) is fully charged over a working pH range of 2-13, binding negatively charged biomolecules.	• Adenovirus process monitoring and quality control • IgM purification monitoring and quality control • Monitoring DNA impurity removal • Monitoring endotoxin removal • HSA Purity	5069-3635
Bio-Monolith DEAE	The diethylaminoethyl bonded phase (Weak Anion-Exchange) offers increased selectivity of biomolecules with negative charge over a working pH range of 3-9.	• Process monitoring and quality control of bacteriophage manufacturing and purification • Process monitoring and quality control of plasmid DNA purification	5069-3636
Bio-Monolith SO_3	The sulfonyl bonded phase (Strong Cation-Exchange) is fully charged over a working pH range of 2-13, binding positively charged biomolecules.	• Fast and high resolution analytical separations of large molecules such as proteins and antibodies • Hemoglobin A1c fast analytics	5069-3637

TIPS & TOOLS

Agilent also offers a Protein A Bio-Monolith column for affinity chromatography. For more information, see pages 434-436.

Column Specifications	
Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2200 psi)
Temperature min/max	Working: 4-40 °C
	Storage: 4-30 °C
Recommended pH	Working range: 2-13
	Cleaning-in-place: 1-14
Materials of construction	Hardware: Stainless steel
	Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith QA: Blue
	Bio-Monolith DEAE: Green
	Bio-Monolith SO_3: Red
Shelf life/expiration date	SO_3, QA, DEAE: 24-36 months

Baseline expansion of a separation of protein standards

Column: **Agilent Bio-Monolith CM15, 5.5 x 15 mm**

Mobile Phase: A: 10 mM Na_2HPO_4, pH 6.0
B: A + 0.5 M NaCl or just 0.5 M Na_2HPO_4, pH 6.0

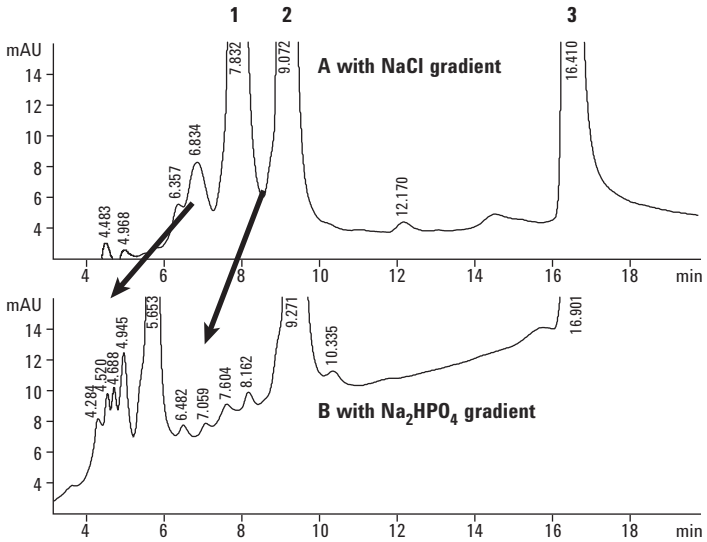
Flow Rate: 2 mL/min

Gradient: 0.5 min hold with mobile phase A followed by a linear gradient to 45% B in 15 min (elapsed time 15.5 min); then 60% B at 15.6 min continued to 20 min. Column flushed with 100% B for 15 min before re-equilibration for the next run.
pH Gradient: A: 5 mM Na_2HPO_4, buffer pH 5.5 and B: 40 mM NA_2HPO_4 (not buffered, pH 8.9). 2% B/min at 1 mL/min for 15 min, followed by a column wash with 90% B for 5 min.

Detector: UV at 220 nm

Sample: One mg each/mL in mobile phase A.
1. RNAse from bovine pancreas (pI 9.6)
2. Cytochrome c from bovine heart (pI 10.37-10.8)
3. Lysozyme from chicken egg (pI 11.35) (0.5 mg)

Instrument: Agilent 1200 SL with diode array detector



B shows a better resolution of protein contaminants.

Bio-Monolith DEAE column monitors phage production during fermentation

Column: **DEAE**
5069-3636
5.2 x 4.95 mm

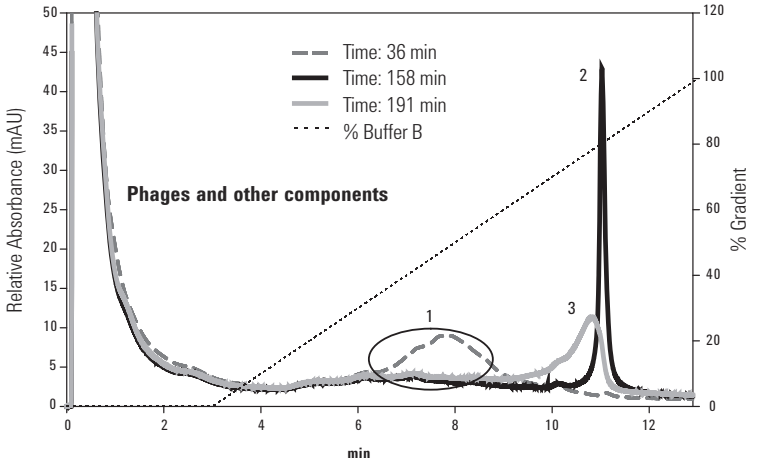
Mobile Phase: A: 125 mM Phosphate buffer, pH 7.0
B: 125 mM Phosphate buffer + 1 M NaCl, pH 7.0

Flow Rate: 1 mL/min

Gradient: 100% buffer A (2.5 min)
0-100% buffer B (10 min)
100% buffer A (2 min)

Detector: UV at 280 nm

Instrument: High pressure gradient HPLC system, Agilent 1200 Infinity LC



As phage proliferation progresses, the genomic DNA (gDNA) concentration increases as the host cells are being lysed. In the late stages of fermentation, gDNA begins to degrade into fragments. These gDNA fragments cannot be easily removed by purification media, therefore it is critical to stop the fermentation cycle prior to the degradation of the genomic DNA. The chromatogram above represents three samples taken from the bioreactor at 36, 158 and 191 minutes. Peak 1 represents phage, media and host cells, peak 2 the intact gDNA and peak 3 the fragmented gDNA.

Size Exclusion Chromatography (SEC)

Accurately determine biomolecule aggregation, fragmentation, and chemical ligation/modification

Size exclusion chromatography (SEC) is a technique for separating proteins, oligonucleotides, and other complex biopolymers by size using aqueous eluents.

Applying SEC to aggregation studies

The size, type, and content of aggregates present in protein biopharmaceuticals can affect both efficacy and formulation – or worse, induce an immunogenic response. Aggregation formations occur through a variety of mechanisms, including disulfide bond formation and non-covalent interactions.

Because the size of protein aggregates, including dimers, is sufficiently different from the protein monomer, you can separate the various forms using SEC. In fact, SEC with UV or light scattering is a standard technique for quantifying protein aggregation.

Applying SEC to quantitation and molecular weight determination

For proteins and other molecules of discreet molecular weight, SEC can be used to detect and quantitate monomers, dimers, aggregates and fragments. SEC can also separate oligonucleotide mixtures.

For biopolymers of varying sizes, like starches and other polysaccharides, SEC can provide data on molecular weight distribution and branching (with the proper detectors).

As a leading manufacturer of SEC columns and instruments for over 30 years, Agilent is continually developing new SEC products that will provide even higher resolution and quicker separations. This section highlights Agilent's broad family of SEC columns for protein biopolymer analysis:

- **Bio SEC-3 and Bio SEC-5 columns** are available in a variety of pore sizes, and are well suited for protein analysis – especially when determining the presence of dimers and aggregates in therapeutic biologicals. Note that 3 µm Bio SEC-3 columns provide higher resolution than our industry-standard 5 µm Bio SEC-5 columns.
- **ProSec 300S columns** work well with globular proteins under high salt conditions.
- **ZORBAX GF-250 and GF-450 columns** are best for preparative SEC of proteins, because of their larger column size and higher flow rates.
- **PL aquagel-OH columns** can be used to analyze biopolymers of broad molecular weights, such as PEGs, oligo- and polysaccharides, starches, and gums.

Size Exclusion Chromatography (SEC)

Application	Agilent Columns	Notes
Peptides, proteins	Agilent Bio SEC-3	Higher resolution and faster separations from 3 µm particles, with 100Å, 150Å, and 300Å pore sizes.
Large biomolecules and samples with multiple molecular weight components	Agilent Bio SEC-5	More pore size options (100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å) to cover a wider range of analytes.
Globular proteins, antibodies	ProSEC 300S	Single column option for protein analysis in high salt conditions.
Proteins, globular proteins	ZORBAX GF-250/450	Higher flow rate capabilities and larger column size for SEC semi-prep and prep.
Low MW polymers and oligomers, oligosaccharides, PEGs, lignosulfonates	2 or 3 PL aquagel-OH • PL aquagel-OH 8 µm • PL aquagel-OH 20 5 µm • PL aquagel-OH MIXED-M 8 µm	The PL aquagel-OH analytical series has a pH range of 2-10, compatibility with organic solvent (up to 50% methanol), mechanical stability up to 140 bar (2030 psi), and low column operating pressures.
Polydisperse biopolymers, polysaccharides, cellulose derivatives	2 or 3 PL aquagel-OH • PL aquagel-OH MIXED-H 8 µm • PL aquagel-OH 60/50/40 8 µm	
Very high MW polymers, hyaluronic acids, starches, gums	PL aquagel-OH 60/50/40 15 µm in series	

Agilent Bio SEC-3

- Exceptional loading capacity, stability, and reproducibility for size-based biomolecule separations
- Sharper peaks, higher resolution, and better protein recovery
- Faster separations than large-particle SEC columns
- Compatibility with most aqueous buffers
- Excellent stability in high-salt and low-salt conditions

Agilent Bio SEC-3 HPLC columns are a breakthrough technology for size exclusion chromatography (SEC). They are packed with spherical, narrowly dispersed 3 µm silica particles coated with a proprietary hydrophilic layer. This thin polymeric layer is chemically bonded to pure, mechanically stable silica under controlled conditions, ensuring a highly efficient size exclusion particle.

Agilent Bio SEC-3 HPLC columns are available in 100Å, 150Å and 300Å pore sizes to accommodate most peptide and protein size exclusion separations.

Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	3 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	3 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	3 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

TIPS & TOOLS

Deactivated/silanized vials have inert surfaces that will not interact with metals, biologicals or proteins, and will not cause pH shifts. Avoid standard polypropylene vials for biological or light-sensitive compounds.

Calibration curves – Bio SEC-3

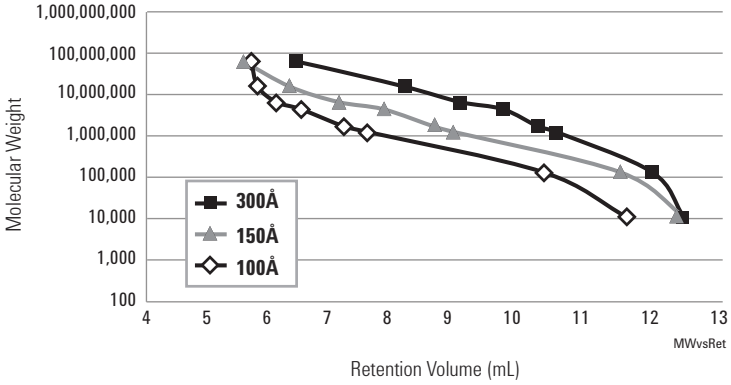
Column: **Bio SEC-3**
7.8 x 300 mm, 3 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

Detector: UV

Proteins	MWt	300Å	150Å	100Å
Thyroglobulin	670000	6.34	5.50	5.63
Gamma globulin	158000	8.03	6.24	5.74
BSA	67000	8.90	7.00	6.03
Ovalbumin	45000	9.57	7.70	6.41
Myoglobin	17000	10.12	8.50	7.10
Ribonuclease A	12700	10.40	8.80	7.46
Vitamin B-12	1350	11.90	11.40	10.20



Intact MAb monomer and dimer separation

Column: **Bio SEC-3, 300Å**
5190-2511
7.8 x 300 mm, 3 µm

Buffer: Sodium phosphate buffer, pH 7.0, 150 mM

Isocratic: 0-100% Buffer A from 0-30 min

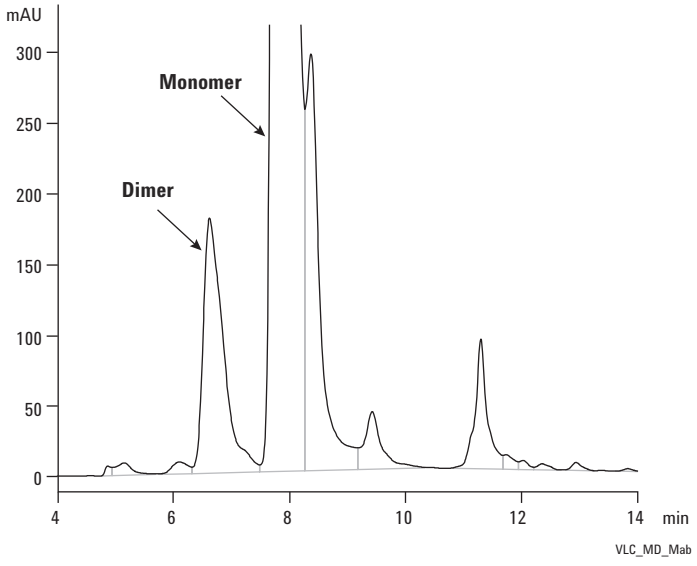
Flow Rate: 1.0 mL/min

Sample: CHO-humanized MAb, 5 mg/mL – intact

Injection: 5 µL

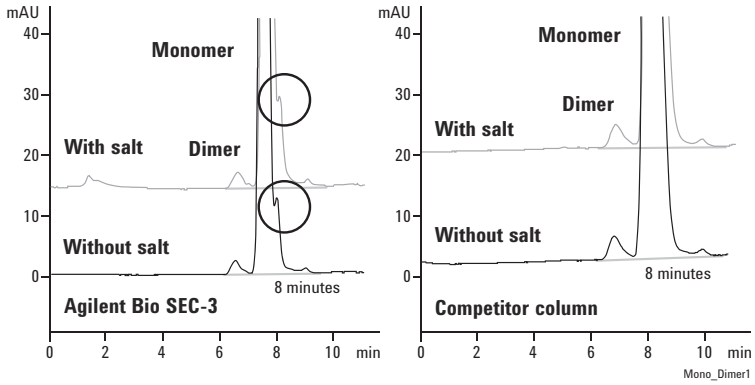
Detector: UV 220 nm

Temperature: Ambient



Comparison of Agilent Bio SEC-3 and competitor column in the analysis of a monoclonal antibody

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Column:	Competitor 7.8 x 300 mm
Mobile Phase:	150 mM sodium phosphate + 100 mM Na sulfate (with salt) 150 mM sodium phosphate (without salt)
Flow Rate:	1.0 mL/min
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



The Agilent Bio SEC-3 column reveals the presence of smaller MW species missed by the competitor column.

Monoclonal Antibody Monomer and Dimer Analysis using Agilent Bio SEC-3 and a Competitor Column

Eluent	Column	Resolution Ratio Monomer:Dimer	Monomer Efficiency	Percentage Dimer
With salt	Agilent	2.04	7,518	0.59
With salt	Competitor	1.88	3,967	0.59
Without salt	Agilent	2.08	7,942	0.60
Without salt	Competitor	1.92	4,164	0.57

Pore Size Choice

The choice of media pore size will influence the resolution in SEC. As the separation is based on differences in molecular size in solution, the sample must be able to permeate the porous structure of the particles – if the pore size is too small, the samples will be excluded from the pores and elute in the void volume of the column, and if too large then, all will be able to fully permeate the particles and so there will be very little separation.

Pore size choice: Proteins

Column A: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 150Å**
5190-2508
4.6 x 300 mm, 3 µm

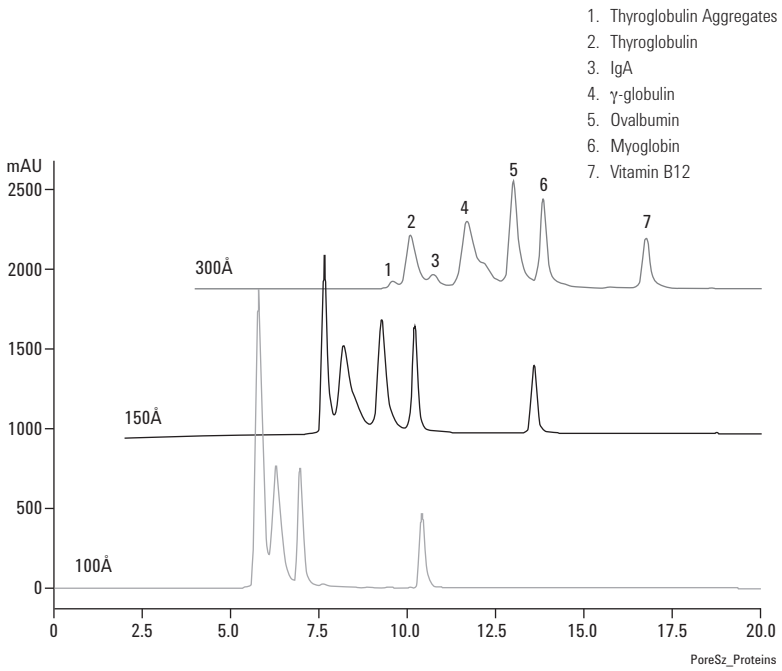
Column C: **Bio SEC-3, 300Å**
5190-2513
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 + 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV, 220 nm

Sample: BioRad Gel Filtration Standards Mix



Pore size choice: Mouse IgG

Column A: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

Column B: **Bio SEC-3, 150Å**
5190-2508
4.6 x 300 mm, 3 µm

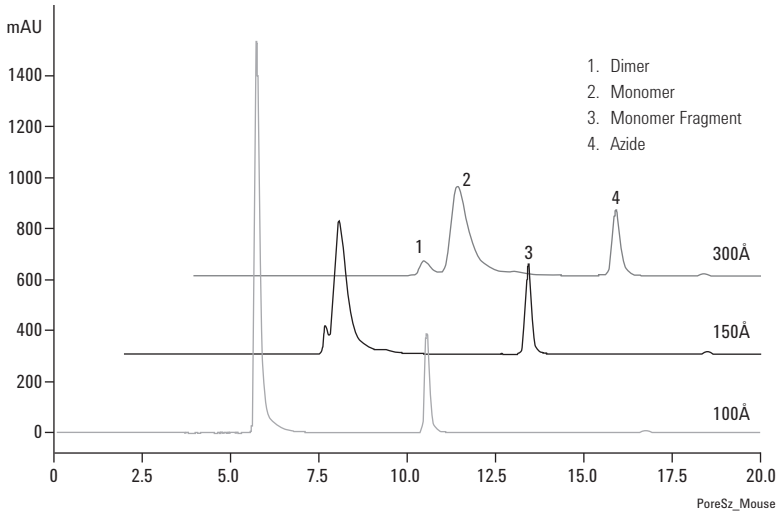
Column C: **Bio SEC-3, 300Å**
5190-2513
4.6 x 300 mm, 3 µm

Mobile Phase: 50 mM Na_2HPO_4, 50 mM NaH_2PO_4 + 0.15 M NaCl, pH 6.8

Flow Rate: 0.35 mL/min

Detector: UV, 220 nm

Sample: Mouse IgG

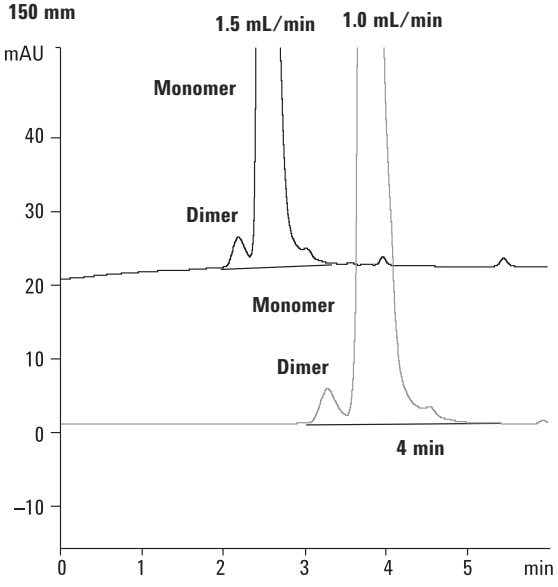


Column Length

Where the separation time is a critical parameter, shorter columns packed with the higher efficiency, 3 µm media are used. With the shorter columns, higher flow rates are used to reduce the analysis time but without compromising the quality of the data – quantitation of monoclonal antibody monomer and dimer.

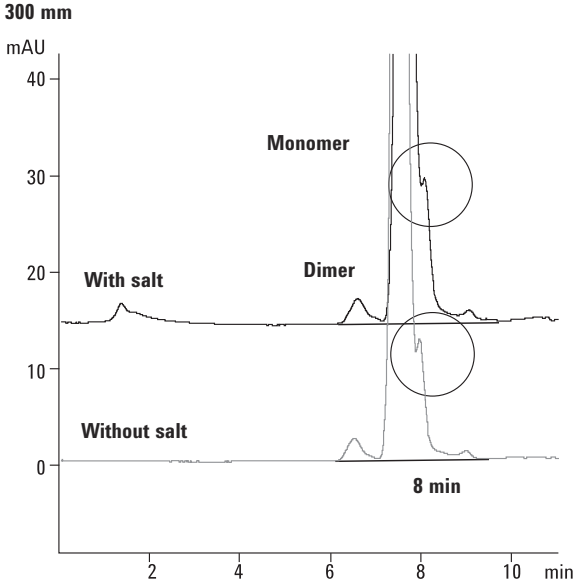
Agilent Bio SEC-3 column length comparison, 150 mm

Column:	**Bio SEC-3, 300Å** **5190-2512** **7.8 x 150 mm, 3 µm**
Mobile Phase:	150 mM sodium phosphate
Flow Rate:	1.0 mL/min (56 bar), 1.5 mL/min (75 bar)
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



Agilent Bio SEC-3 column length comparison, 300 mm

Column:	**Bio SEC-3, 300Å** **5190-2511** **7.8 x 300 mm, 3 µm**
Mobile Phase:	150 mM sodium phosphate + 100 mM Na sulfate (with salt) 150 mM sodium phosphate (without salt)
Flow Rate:	1.0 mL/min
Detector:	UV, 220 nm
Sample:	MAb (2 mg/mL)



Agilent Bio SEC-3

Size (mm)	Particle Size (µm)	Bio SEC-3 100Å USP L33	Bio SEC-3 150Å USP L33	Bio SEC-3 300Å USP L33
7.8 x 300	3	5190-2501	5190-2506	5190-2511
7.8 x 150	3	5190-2502	5190-2507	5190-2512
4.6 x 300	3	5190-2503	5190-2508	5190-2513
4.6 x 150	3	5190-2504	5190-2509	5190-2514
7.8 x 50, Guard	3	5190-2505	5190-2510	5190-2515

Agilent Bio SEC-5

- Maximum recovery for a broad range of size-based, biomolecule separations
- Outstanding reproducibility and column lifetime
- Excellent stability, even under high-pH, high-salt, and low-salt conditions
- Compatibility with most aqueous buffers

Agilent Bio SEC-5 HPLC columns are packed with 5 µm silica particles coated with a proprietary, neutral, hydrophilic layer for maximum efficiency and stability. Our specially designed packing also provides high pore volume, improving both peak capacity and resolution.

Bio SEC-5 columns are available in 5 µm particles with 100Å, 150Å, 300Å, 500Å, 1000Å, and 2000Å nominal pore sizes.

Column Specifications

Pore Size	Particle Size	MW Range	pH Range	Max Pressure	Flow Rate
100Å	5 µm	100-100,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
150Å	5 µm	500-150,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
300Å	5 µm	5,000-1,250,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
500Å	5 µm	15,000-5,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
1000Å	5 µm	50,000-7,500,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)
2000Å	5 µm	>10,000,000	2-8.5	240 bar, 3500 psi	0.1-1.25 mL/min (7.8 mm id)
					0.1-0.4 mL/min (4.6 mm id)

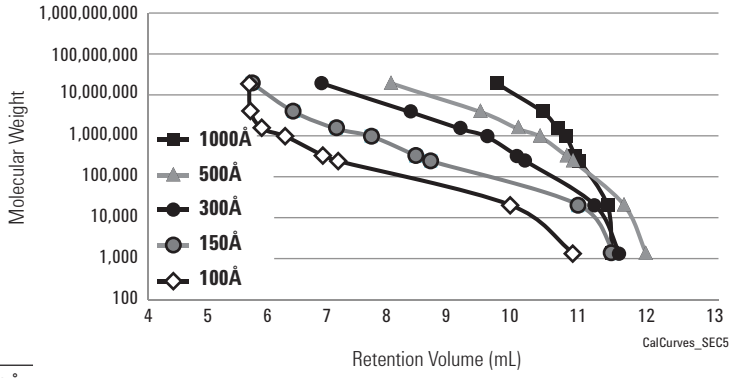
Calibration curves – Bio SEC-5

Column: **Bio SEC-5**
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

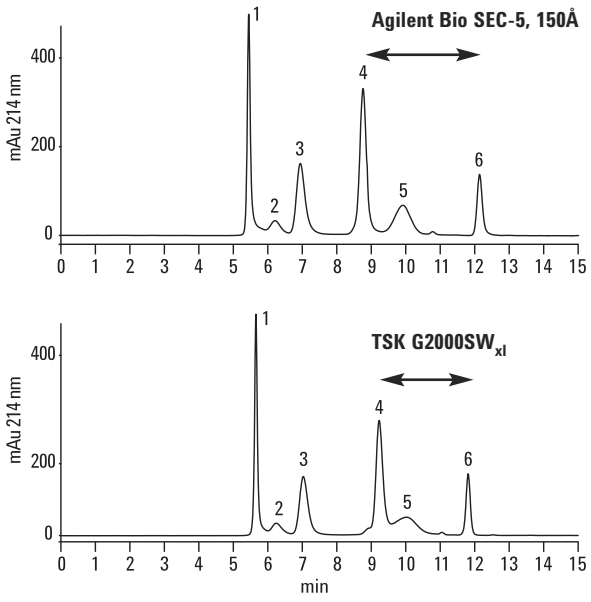
Flow Rate: 1.0 mL/min

Detector: UV, 214 nm



		Retention Volume				
Proteins	**MW**	**1000Å**	**500Å**	**300Å**	**150Å**	**100Å**
Thyroglobulin	670000	10.07	8.23	7.03	5.82	5.77
Gamma globulin	158000	10.88	9.80	8.57	6.55	5.79
BSA	67000	11.13	10.44	9.44	7.29	6.00
Ovalbumin	45000	11.28	10.83	9.89	7.90	6.40
Myoglobin	17000	11.44	11.28	10.42	8.66	7.05
Ribonuclease A	12700	11.52	11.41	10.58	8.93	7.32
Vitamin B-12	1350	12.00	12.59	11.78	11.49	10.30

Side-by-side comparison



Column: **Bio SEC-5**
5190-2521
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Na phosphate, pH 7.0

Flow Rate: 1.0 mL/min

Detector: UV, 214 nm

1. Thyroglobulin, 5.43 min
2. BSA dimer, 6.19 min
3. BSA monomer, 6.93 min
4. Ribonuclease A, 8.74 min
5. Poly-DL-alanine (1-5 kDa), 9.90 min
6. Uracil, 12.13 min

1. Thyroglobulin, 5.64 min
2. BSA dimer, 6.23 min
3. BSA monomer, 7.02 min
4. Ribonuclease A, 9.22 min
5. Poly-DL-alanine (1-5 kDa), 10.02 min
6. Uracil, 11.81 min

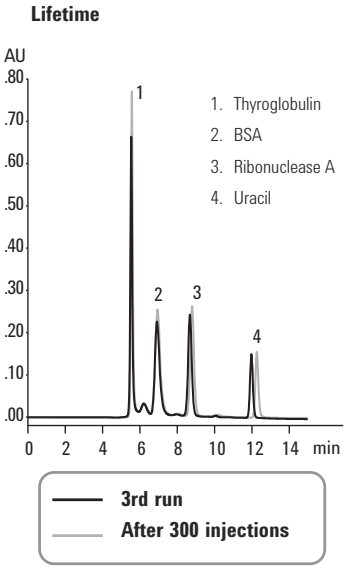
Separation of a protein mixture on an Agilent Bio SEC-5 HPLC column and a Tosoh TSK-Gel column. Notice the sharper peaks and better resolution on the Agilent Bio SEC-5 HPLC column.

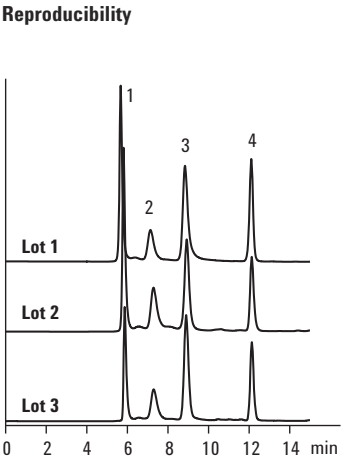
Exceptional lifetime, and lot-to-lot reproducibility

Column: **Bio SEC-5, 150Å**
5190-2521
7.8 x 300 mm, 5 µm

Mobile Phase: 150 mM Phosphate Buffer, pH 7.0

The four protein mixture shows excellent retention time reproducibility over 300 injections and on three columns from different manufacturing lots.





Comparison between Agilent Bio SEC-3 and Agilent Bio SEC-5

Analysis of monoclonal antibody

Column: **Bio SEC-3, 300Å**
5190-2511
7.8 x 300 mm, 3 µm

Column: **Bio SEC-5, 300Å**
5190-2526
7.8 x 300 mm, 5 µm

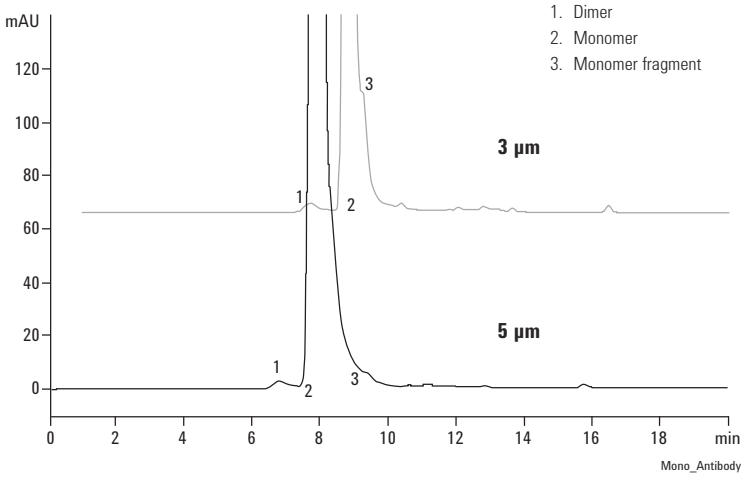
Mobile Phase: 150 mM Sodium Phosphate, pH 7

Flow Rate: 1 mL/min

Detector: UV @ 220 nm

Sample: Humanized monoclonal antibody

The 3 µm column gives better separation



Agilent Bio SEC-5

Size (mm)	Particle Size (µm)	Bio SEC-5 100Å USP L33	Bio SEC-5 150Å USP L33	Bio SEC-5 300Å USP L33	Bio SEC-5 500Å USP L33	Bio SEC-5 1000Å USP L33	Bio SEC-5 2000Å USP L33
7.8 x 300	5	5190-2516	5190-2521	5190-2526	5190-2531	5190-2536	5190-2541
7.8 x 150	5	5190-2517	5190-2522	5190-2527	5190-2532	5190-2537	5190-2542
4.6 x 300	5	5190-2518	5190-2523	5190-2528	5190-2533	5190-2538	5190-2543
4.6 x 150	5	5190-2519	5190-2524	5190-2529	5190-2534	5190-2539	5190-2544
7.8 x 50, Guard	5	5190-2520	5190-2525	5190-2530	5190-2535	5190-2540	5190-2545

TIPS & TOOLS

The Agilent rack can be used to optimize your 1290 Infinity LC for ultra-low dispersion, which can enhance performance of high-efficiency columns. Further information can be found in application note 5990-9502EN at **www.agilent.com/chem/library**

ProSEC 300S

- Mechanically robust silica particles that do not bleed during use
- Single column with extended linear resolving range
- Column dimensions for use with multi-detector systems

The Agilent ProSEC 300S column is specifically designed as a single column solution for globular protein analysis. The pore size selection and optimization provides an extended linear resolving range so that this single column can be used for analysis across the full range of globular proteins.

The particles are extremely robust and do not fragment during use to leach particulates. This gives exceptionally stable baselines making this column an ideal choice for use with light scattering detectors.

Two column dimensions, 7.5 mm id and 4.6 mm id, to suit multi-detector size exclusion chromatography provide an option for the analysis of small masses.

ProSEC 300S Column Specifications

Bonded Phase	Pore Size	Particle Size	Protein MW Range	pH Range	Flow Rate	Max Pressure
ProSEC 300S	300Å	5 µm	1,500-800,000	2-7.5	<1.5 mL/min (7.5 mm id)	250 bar, 3700 psi
					<0.5 mL/min (4.6 mm id)	

ProSEC 300S

Dimensions	Particle Size (µm)	Part No.
4.6 x 250	5	PL1547-5501
7.5 x 300	5	PL1147-6501
Guard Columns		
4.6 x 50	5	PL1547-1501
7.5 x 50	5	PL1147-1501

Calibration of the ProSEC 300S column with globular proteins

Mobile Phase: 50 mM KH_2PO_4-K_2HPO_4 (@ pH 6.8) containing 0.3 M NaCl

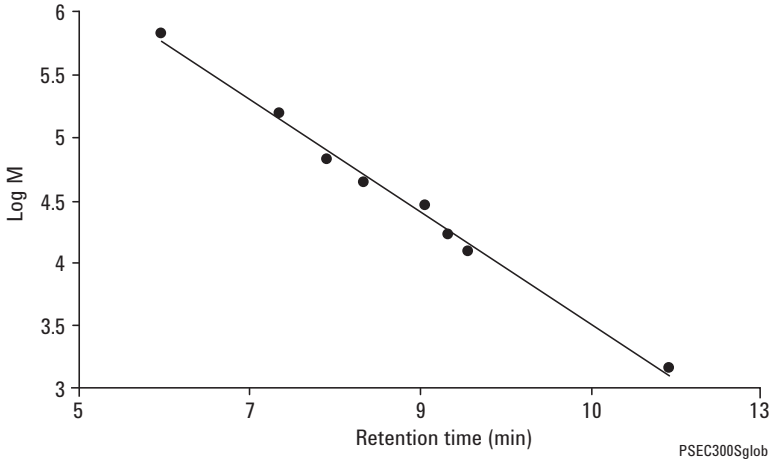
Flow Rate: 1.0 mL/min

Detector: UV, 280 nm

Sample: Protein samples

Molecular weights of the proteins

Mw/Daltons	Protein
670,000	Thyroglobulin
155,000	γ-Globulin
66,430	Bovine serum albumin
44,287	Ovalbumin
29,000	Carbonic anhydrase
16,700	Myoglobulin
12,384	Cytochrome c
1,423	Bacitracin



Analysis of Bovine Serum Albumin by light scattering using ProSEC 300S columns

Column: ProSEC 300S
PL1147-6501
7.5 x 300 mm, 5 µm

Mobile Phase: Water + 120 mM NaCl, 2.7 mM KCl, 10 mM NaH_2PO_4

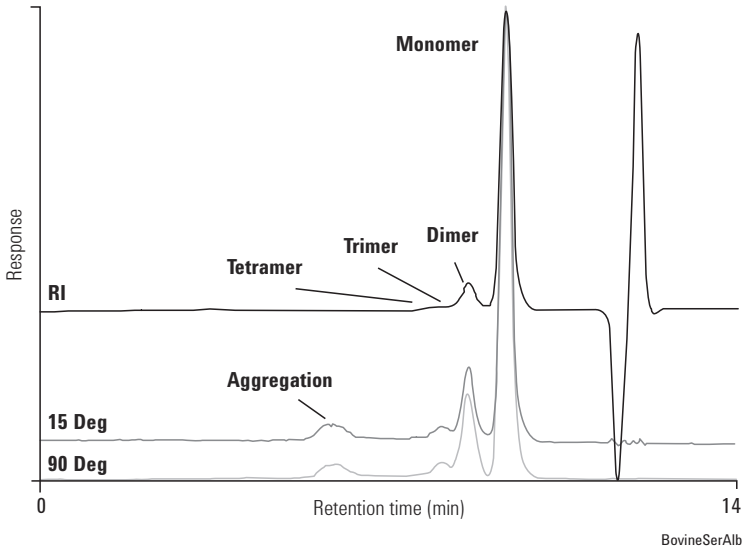
Flow Rate: 1.0 mL/min

Detector: Differential refractive index + PL-GPC 50 Dual Angle Light Scattering Detector

Sample: Bovine serum albumin

Molecular Weights

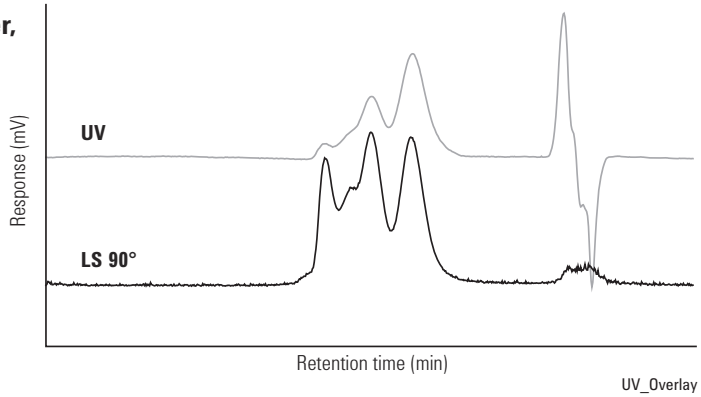
Monomer	66,900 Daltons, 88.5%
Dimer	34,900 Daltons (2.02 x monomer molecular weight), 9.8%
Trimer	197,000 Daltons (2.94 x monomer molecular weight), 1.2%
Tetramer	279,300 Daltons (5.17 x monomer molecular weight), 0.5%



Overlay of differential refractive index and dual angle light scattering sample.

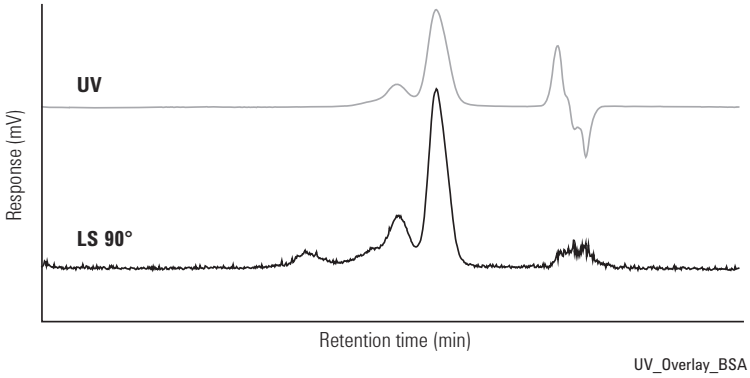
Overlay of UV and light scattering 90° for a sample of γ-globulins, illustrating monomer, dimer, and trimer peaks

Column:	**ProSEC 300S PL1147-6501 7.5 x 300 mm, 5 µm**
Mobile Phase:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0
Flow Rate:	1.0 mL/min
Temperature:	5 °C
Detector:	UV at 310 nm + PL-GPC 50 Dual Angle Light Scattering Detector
Sample:	Proteins



Overlay of UV and light scattering 90° for a sample of BSA, illustrating monomer, dimer, trimer and aggregate peaks

Column:	**ProSEC 300S PL1147-6501 7.5 x 300 mm, 5 µm**
Mobile Phase:	0.1 M KH_2PO_4 containing 0.3 M NaCl, pH 8.0
Flow Rate:	1.0 mL/min
Temperature:	5 °C
Detector:	UV at 310 nm + PL-GPC 50 Dual Angle Light Scattering Detector
Sample:	Proteins



ZORBAX GF-250 and GF-450 Gel Filtration Columns

GF-250 Gel Filtration Columns

- High efficiency and reproducibility with short analysis time
- Semi-prep and prep column dimensions
- Compatible with organic modifiers and denaturants
- Wide usable pH range (3-8)

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

Column Specifications

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

Specifications represent typical values only

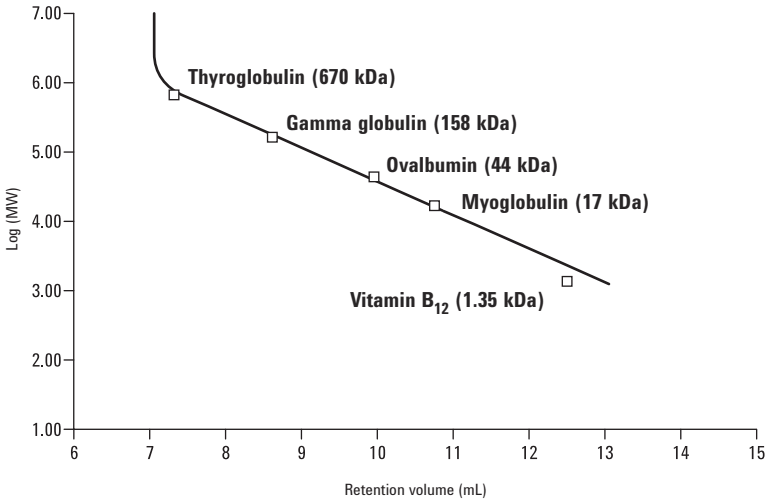
Retention volume versus log (MW) for the Bio-Rad standards separated on an Agilent ZORBAX GF-250 column

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

Mobile Phase: 200 mM Sodium phosphate, pH 7.0

Temperature: Ambient

Detector: UV, 254 nm



Separations of proteins on preparative columns

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

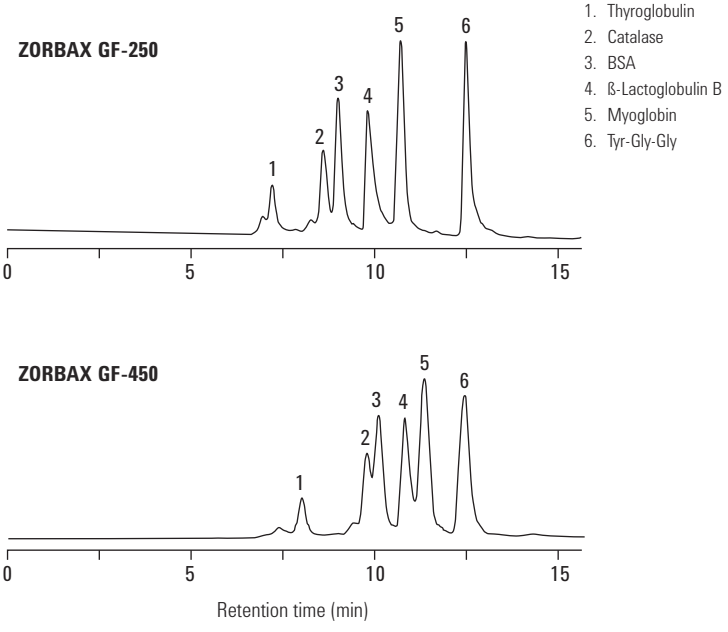
Column: **ZORBAX GF-450**
884973-902
9.4 x 250 mm, 6 µm

Mobile Phase: 0.2 M Na_2HPO_4, pH 7.0

Flow Rate: 5.0 mL/min

Detector: UV, 280 nm

Sample: 200 µL



ZORBAX GF-250 (USP L33) and GF-450 (USP L35) Gel Filtration Columns

Hardware	Description	Size (mm)	Particle Size (µm)	Part No.
	GF-250, 150Å	9.4 x 250	4	884973-901
	GF-250, 150Å	4.6 x 250	4	884973-701
	GF-450, 300Å	9.4 x 250	6	884973-902
Guard Columns (hardware required)				
P	GF-250 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
ZGC	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
P	GF-450 Diol, Guard Cartridge, 2/pk	9.4 x 15	6	820675-111
ZGC	GF-250 Diol, Guard Cartridge, 4/pk	4.6 x 12.5	6	820950-911
P	Prep Guard Hardware Kit			840140-901
ZGC	Guard Hardware Kit			820999-901
PrepHT Columns				
PL	PrepHT GF-250, 150Å	21.2 x 250	6	877974-901
PL	PrepHT GF-450, 300Å	21.2 x 250	6	877974-910
PL	PrepHT Endfittings, 2/pk			820400-901
PL	PrepHT GF-250, Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-911
PL	PrepHT GF-450, Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-911
PL	Guard Cartridge Hardware			820444-901

Bio-Monolith Protein A Column, 5069-3639

Affinity Chromatography

Affinity chromatography is a powerful technique which takes advantage of highly specific molecular interactions, frequently between specific proteins (e.g. antigen/antibody). Agilent offers several specialty affinity products, a monolithic Protein A column for the isolation and quantitation of IgG and a series of Multiple Affinity Removal Systems for the elimination of high abundance proteins in biological samples.

Agilent Bio-Monolith Protein A HPLC Columns

- Designed for the analytical separation of all IgG (human and mouse), except for IgG class3
- Flow-rate independent separations; no diffusion, no pores and no void volume make transport between mobile and stationary phase very rapid
- Extremely fast separations speed up method development time and decrease costs
- Locking in method parameters takes significantly less time and buffer

Agilent Bio-Monolith Protein A HPLC columns are part of the Agilent Bio-Monolith column family. Protein A Bio-Monolith columns are compatible with HPLC and preparative LC systems, including Agilent 1100 and 1200 HPLC systems.

TIPS & TOOLS

For information on Ion-Exchange Bio-Monolith columns, turn to pages 412-415.

Column Specifications	
Dimensions	5.2 mm x 4.95 mm
Column volume	100 µL
Maximum pressure	150 bar (15 MPa, 2200 psi)
Temperature min/max	Working: 4-40 °C
	Storage: 4-30 °C
Recommended pH	Working range: 2-13
	Cleaning-in-place: 1-14
Materials of construction	Hardware: Stainless steel
	Packing: poly(glycidyl methacrylate-co-ethylene dimethacrylate) highly porous monolith
Color ring identifier	Bio-Monolith Protein A: White
Shelf life/expiration date	Protein A: 12 months

Bio-Monolith Protein A

Column	Description	Key Applications	Part No.
Bio-Monolith Protein A	The Protein A affinity column is designed for the analytical separation of all IgG (human and mouse), except for IgG class3.	• Quantitative determination of IgG (fermentation titer calculation)	5069-3639

TIPS & TOOLS

Further information can be found in the following application note:

Rapid Human Polyclonal IgG Quantification Using the Agilent Bio-Monolith Protein A HPLC Column (publication # 5989-9733EN)

www.agilent.com/chem/library

Rapid human polyclonal IgG quantification using the Agilent Bio-Monolith Protein A HPLC column

Column: **Protein A**
5069-3639
5.2 x 4.95 mm

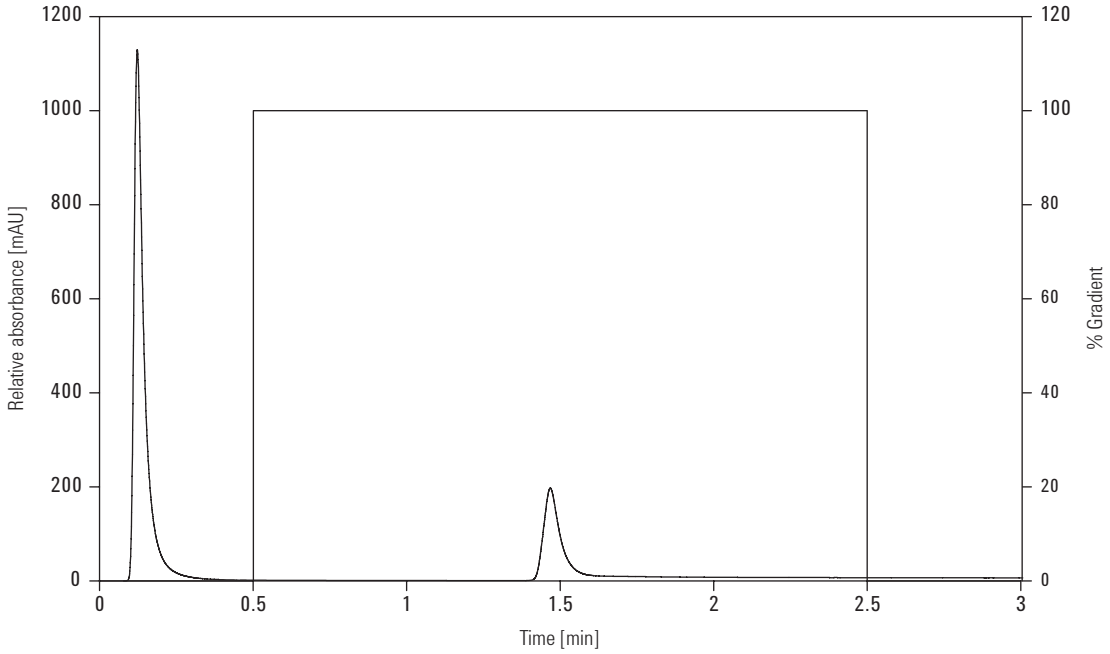
Mobile Phase: PBS buffer, pH 7.4
0.5 M acetic acid, pH 2.6

Flow Rate: 1 mL/min

Gradient: Stepwise gradient: 100% buffer A-100% buffer B-100% buffer A (0.5 min each step)

Detector: A high pressure gradient HPLC system, Agilent 1200 Infinity LC - UV at 280 nm

Sample: Human Plasma diluted with binding buffer (PBS buffer, pH 7.4)



The selectivity of the Bio-Monolith Protein A column for the IgG from human plasma. IgG binds to protein A, a 100% buffer B step gradient is applied, and IgG elutes at 1.5 min.

Key:

Lane 1: Whole serum prior to separation

Lane 2: IgG standard

Lane 3: Peak 1 (flow-through fraction)

Lane 4: Peak 2 (Protein A-bound fraction; i.e. IgG1 and IgG2)

SDS PAGE analysis of fractions from the separation.

Agilent Protein Fractionation System and Proteomics Reagents

- LC/MS analysis of biological samples
- Preparation for electrophoretic analysis
- Sample preparation for biomarker discovery
- Instrument and workflow validation
- Cost-effective immunodepletion
- Sample desalting, concentration, and fractionation

In order to more easily isolate and identify proteins in biological samples, such as serum, plasma, and cerebro-spinal fluid (CSF), the Agilent Multiple Affinity Removal System is designed to chromatographically eliminate interfering high-abundance proteins from biological samples. Removal of these abundant proteins improves the subsequent LC/MS and electrophoretic analysis of the sample by effectively expanding the dynamic range.

For sample fractionation and desalting, the Agilent mRP-C18 High-Recovery Protein column is designed to simultaneously desalt, concentrate, and fractionate in one easy step with extremely high recovery of samples as compared to conventional RP HPLC columns that are fully compatible with LC/MS analysis.

In addition, validated reagents for sample preparation in biomarker discovery and other proteomics applications are also available, including a complex standard, and proteomics grade trypsin. For your convenience, these reagents are fully compatible with Agilent LC/MS methods and require no additional sample pretreatments.

Large volume requirements and custom column dimensions can also be addressed with our custom configurations.

Multiple Affinity Removal System

Multiple Affinity Removal System

The Multiple Affinity Removal System from Agilent enables the identification and characterization of high-value, low abundant proteins and biomarkers found in serum, plasma, and other biological fluids.

The Multiple Affinity Removal System reproducibly and specifically removes up to 14 high abundant proteins found in human biological fluids and 3 high abundant proteins found in mouse biological fluids.

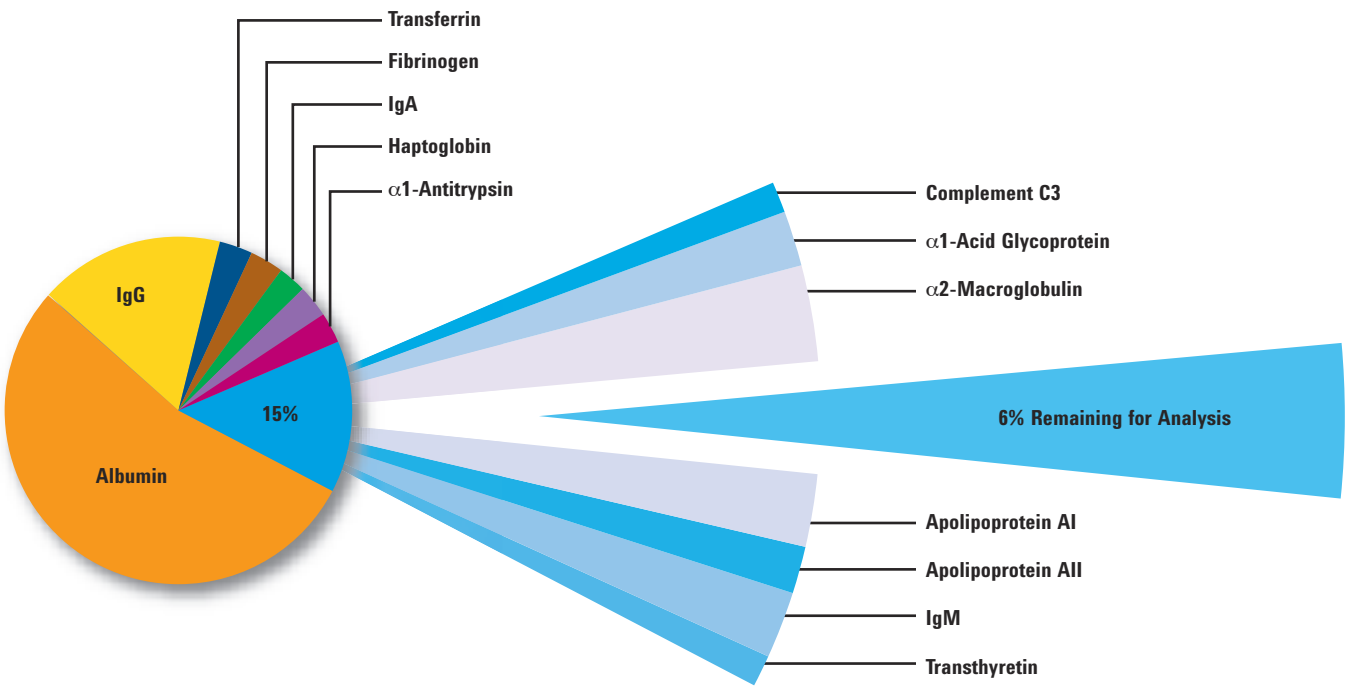
The Multiple Affinity Removal System is available in a variety of LC column dimensions and in spin cartridge format. When combined with Agilent's optimized buffers, convenient spin filters and concentrators, the Agilent Multiple Affinity Removal System creates an automated, integrated depletion solution compatible with most LC instruments (columns), and bench top centrifuges (spin cartridges).

Samples depleted using the Multiple Affinity Removal System are ready for downstream analyses such as 2-D gel electrophoresis, LC/MS, and other analytical techniques.

Multiple Affinity Removal System Selection Guide

Product	Proteins Removed	Total Protein Removed	Dimension	Load Capacity	Part No.
MARS Human-14	Albumin, IgG, antitrypsin, IgA, transferrin, haptoglobin, fibrinogen, alpha2-macroglobulin, alpha1-acid glycoprotein, IgM, apolipoprotein AI, apolipoprotein AII, complement C3, transthyretin	94%	Spin Cartridge	8 - 10 µL	5188-6560
			4.6 x 50 mm	20 µL	5188-6557
			4.6 x 100 mm	40 µL	5188-6558
			10.0 x 100 mm	250 µL	5188-6559
MARS Human-7	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin, fibrinogen	88-92%	Spin Cartridge	12 - 14 µL	5188-6408
			4.6 x 50 mm	30 - 35 µL	5188-6409
			4.6 x 100 mm	60 - 70 µL	5188-6410
			10.0 x 100 mm	250 - 300 µL	5188-6411
MARS Human-6	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	7 - 10 µL	5188-5230
			4.6 x 50 mm	15 - 20 µL	5185-5984
			4.6 x 100 mm	30 - 40 µL	5185-5985
MARS Human-6 High Capacity	Albumin, IgG, IgA, transferrin, haptoglobin, antitrypsin	85-90%	Spin Cartridge	14 - 16 µL	5188-5341
			4.6 x 50 mm	30 - 40 µL	5188-5332
			4.6 x 100 mm	60 - 80 µL	5188-5333
			10.0 x 100 mm	up to 340 µL	5188-5336
MARS Human-2	Albumin, IgG	69%	Spin Cartridge	50 µL	5188-8825
			4.6 x 50 mm	100 µL	5188-8826
MARS Human-1	Albumin	50-55%	Spin Cartridge	65 µL	5188-5334
			4.6 x 50 mm	130 µL	5188-6562
MARS Mouse-3	Albumin, IgG, transferrin	80%	Spin Cartridge	25 - 30 µL	5188-5289
			4.6 x 50 mm	37 - 50 µL	5188-5217
			4.6 x 100 mm	75 - 100 µL	5188-5218

Illustration of high abundance proteins removed by Agilent Multiple Affinity Removal Columns and Spin Cartridges



TIPS & TOOLS

Learn more about Agilent's complete services portfolio at **www.agilent.com/chem/services**

Multiple Affinity Removal System Starter Kits

The LC Column and Spin Cartridge Reagent Starter Kits include all the required supplies to use with Multiple Affinity Removal System. These buffers provide optimal conditions for column longevity and sample reproducibility.

- The kits provide enough Buffer A and Buffer B for approximately 200 sample depletions using the 4.6 x 50 mm LC columns, approximately 100 sample depletions using the 4.6 x 100 mm LC columns and 200 spin cartridge uses.
- Buffer A, the loading buffer, minimizes protein-protein interactions, allowing low abundant proteins often bound to high abundant proteins to pass through the column, while the targeted high abundant proteins bind to their associated antibodies.
- Buffer B, the elution buffer, then disrupts the antibody-protein interaction eluting the high abundant proteins off the column.

Multiple Affinity Removal System Starter Kits

Description	Part No.
LC Column Reagent Starter Kit Includes:	5185-5986
Buffer A, 1 L, for loading, washing, and equilibrating, qty 2	5185-5987
Buffer B, 1 L, for eluting	5185-5988
0.22 µm cellulose acetate, 25/pk, qty 2	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Multiple Affinity Removal Spin Cartridge Reagent Kit Includes:	5188-5254
Buffer A, 1 L, for loading, washing, and equilibrating	5185-5987
Buffer B, 1 L, for eluting	5185-5988
Spin filters, 0.22 µm cellulose acetate, 25/pk, qty 2	5185-5990
Spin concentrators, 5K MWCO, 4 mL, 25/pk	5185-5991
Luer-Lok adapters, 2/pk	5188-5249
Plastic syringe, 5 mL, Luer-Lok, 2/pk	5188-5250
Microtube, 1.5 mL, screw top, 100/pk, qty 6	5188-5251
Caps and plugs, 6/pk	5188-5252
PTFE needles, Luer-Lok, 10/pk	5188-5253
High concentration sample dilution buffer, 50 mL	5188-8283

LC Column Reagent Starter Kit, 5185-5986

Luer-Lok adapters, 5188-5249

Luer-Lok syringe, 5188-5250

Luer-Lok needles, 5188-5253

mRP-C18 High-Recovery Protein Column, 4.6 x 50 mm, 5188-5231

mRP-C18 High-Recovery Protein Columns

The mRP (macroporous reversed-phase) C18 High-Recovery Protein column is designed for high recovery, high resolution separation, fractionation, and simultaneous desalting of complex protein samples (like immunodepleted serum or plasma proteins).

- Greater than 95-99% protein sample recovery has been observed with immunodepleted serum using the Agilent Multiple Affinity Removal System – LC column
- Can load up to 380 µg of total protein mass without reducing chromatographic resolution of the proteins
- Column packed with macroporous C18-bonded ultrapure 5 µm particle silica designed to reduce or eliminate strong adsorption of proteins
- Maximum operating pressure of 250 bar (4000 psi)
- Compatible with water and all common organic solvents

mRP-C18 High-Recovery Protein Columns

Description	Protein Load Capacity	Part No.
mRP-C18, 0.5 x 100 mm	10 ng - 5 µg	5188-6510
mRP-C18, 2.1 x 75 mm	8 - 85 µg	5188-6511
mRP-C18, 4.6 x 50 mm	40 - 380 µg	5188-5231

Proteomics Reagents for LC/MS Analysis

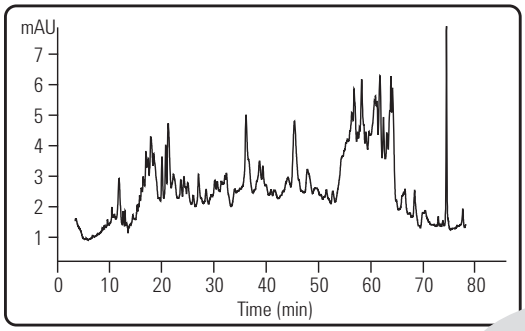
The Agilent Complex Proteomics Standard is a soluble Pfu protein extract containing over 1,500 proteins. Together with our TPCK-treated proteomics grade trypsin this is an ideal combination for workflow validation in LC/MS biomarker discovery and other proteomic studies.

Proteomics Reagents for LC/MS Analysis

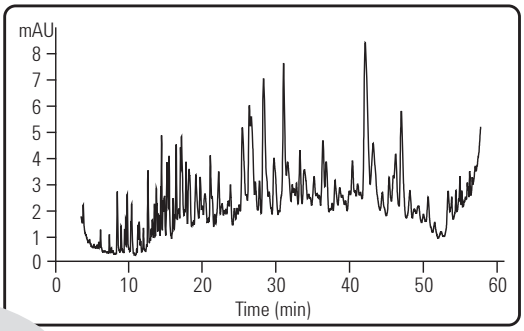
Description	Part No.
Complex Proteomics Standard	400510
Proteomics Grade Trypsin	204310

Protein Fractionation of Complex Samples on the mRP Column

mRP-C18, 4.6 x 50 mm

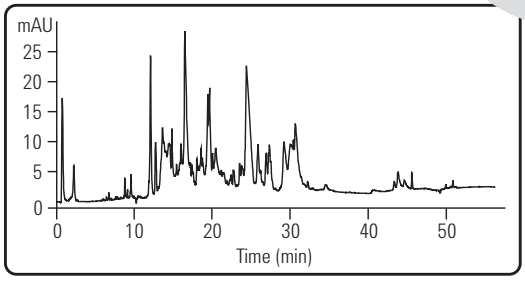


HeLa Membrane Prep

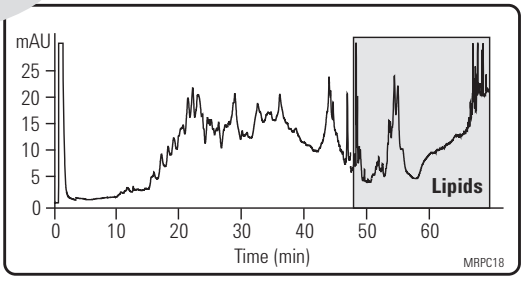


HeLa Cell Lysate (352 µg)

Highest Recovery



"Top-6" Depleted Human Serum



Human Brain Membrane Lipid Raft Prep (500 µg)

mRP-C18 High-Recovery Protein Column, 0.5 x 100 mm, 5188-6510

Method Development

ZORBAX Column Methods

This ZORBAX Column Selection Strategy for Proteins and Peptides provides some critical details on method development for proteins or polypeptides.

Choose the Initial Column and Conditions for Peptides, Polypeptides, and Proteins

Peptides, Polypeptides, Proteins MW <50 kDa	Peptides, Polypeptides, Proteins MW >50 kDa

Initial Bonded Phase

StableBond 300SB-C8

300SB columns are wide-pore columns with unbeatable lifetime in TFA-containing mobile phases. This makes them an ideal first choice for separations of peptides and proteins.

- C8 is an excellent starting bonded phase because of its moderate hydrophobicity
- C18 and C8 are generally selected for peptides and protein digests but can also be used for proteins
- C3, C4 and CN are generally selected for larger, hydrophobic polypeptides and proteins but can also be used for peptides
- PLRP-S when chemical and thermal stability are needed

Poroshell 300SB-C18

Poroshell 300SB columns use an innovative particle technology to deliver rapid protein separations. Short anaysis times with efficient peaks are easily obtained with Poroshell columns.

- C18 is a good starting bonded phase choice with Poroshell for most peptides, polypeptides and proteins because the retention is maximized
- C8 is generally selected for moderate size proteins but can be used with polypeptides or very large proteins
- C3 is generally selected for antibodies or large proteins but can be used for peptides and polypeptides

Initial Separation Conditions

Column:	**StableBond 300SB-C8** **4.6 x 150 mm, 3.5 or 5 µm** **883995-906** **863973-906**
Mobile Phase:	A: 95% H_2O: 5% ACN with 0.1% TFA B: 5% H_2O: 95% ACN with 0.085% TFA
Gradient:	0-60% B in 60 min
Temperature:	35-40 °C
Flow Rate:	1 mL/min

Column:	**Poroshell 300SB-C18** **2.1 x 75 mm, 5 µm** **660750-902**
Mobile Phase:	A: 95% H_2O: 5% ACN with 0.1% TFA B: 5% H_2O: 95% ACN with 0.085% TFA
Gradient:	0-60% B in 60 min
Temperature:	35-40 °C
Flow Rate:	2 mL/min

Start at Low pH with Simple Aqueous/Organic Gradient

Typically, a water/acetonitrile with 0.1% TFA gradient is used to elute all components of interest. A typical high resolution gradient on a 300Å pore size column requires 30-50 min. A Poroshell column requires a shorter analysis time and a higher flow rate and still provides exceptional resolution. To improve resolution, increase the gradient time, decrease column length, or increase flow rate.

Optimize Sample Solubility

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

Solvent Choices to Solubilize Proteins and Peptides

Water/phosphate Buffer
Dilute acid (TFA, Acetic Acid or HCl)
Neutral pH, 6-8 M guanidine-HCl or isthiocyanate
5% HOAc/6 M urea
Dilute acid + aqueous/organic solvents (ACE, MeOH, THF)
Dilute base (ammonium hydroxide)
DMSO or 0.1%-1% in DMSO
Formamide

Weakest
↓
Strongest

Increase the Temperature

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

StableBond 300SB - up to 80 °C

Poroshell 300SB - up to 80 °C

Optimize Mobile Phase pH
Try Mid and High pH if Low pH does not work

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

Column:	**ZORBAX 300Extend-C18 4.6 x 150 mm, 5 µm 773995-902**	Gradient:	5-60% B in 30 min
		Temperature:	25-30 °C (<60 °C)
Mobile Phase:	A: 20 mM NH_4OH in H_2O B: 20 mM NH_4OH in 80% ACN	Flow Rate:	1 mL/min

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

Low pH		Mid and High pH
MW <50 kDa **ZORBAX 300SB-C8** **4.6 x 150 mm, 3.5 µm** 863973-906	**MW >50 kDa** **Poroshell 300SB-C18** **2.1 x 75 mm, 5 µm** 660750-902	**MW <25 kDa** **ZORBAX 300Extend-C18** **2.1 x 150 mm, 3.5 µm** 763750-902

Reversed-Phase LC/MS Methods

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

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

Analytical LC/MS applications – 2.1 mm id columns provide good sensitivity when sample size is not limited. With Poroshell columns, smaller 1 mm column ids are used.

Low pH		Mid and High pH
MW <50 kDa **ZORBAX 300SB-C8** **2.1 x 150 mm, 3.5 µm** 863750-906	**MW <1000 kDa** **Poroshell 300SB-C18** **1.0 x 75 mm, 5 µm** 661750-902	**MW <25 kDa** **ZORBAX 300Extend-C18** **2.1 x 150 mm, 3.5 µm** 763750-902

High Sensitivity/Proteomics Applications

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

Bio Ion-Exchange Column Methods

Bio MAb Monoclonal Antibodies	Cation-Exchange (SCX, WCX) Monoclonal Antibodies, Proteins, Peptides, and Glycoproteins	Anion-Exchange (SAX, WAX) Oligonucleotides, Proteins

Recommended Initial Conditions

	Bio MAb Monoclonal Antibodies	Cation-Exchange (SCX, WCX) Monoclonal Antibodies, Proteins, Peptides, and Glycoproteins	Anion-Exchange (SAX, WAX) Oligonucleotides, Proteins
Mobile Phase:	A: 20 mM sodium phosphate, pH 5.5 B: Buffer A plus 400 mM NaCl	A: 20 mM sodium phosphate, pH 5.0 for WCX or pH 6.0 for SCX B: Buffer A plus 500 mM NaCl	A: 10 mM Tris buffer, pH 8 for WAX or pH 9.0 for SAX B: Buffer A plus 400 mM NaCl
Gradient:	10-35% B in 50 min	1-100% B in 30 min for 50 mm columns; 60 min for 250 mm columns	1-100% B in 30 min for 50 mm columns and 60 min for 250 mm columns
Sample Size:	2 mg/mL; 5 µL injection	2 mg/mL; 5 µL injection	2 mg/mL; 5 µL injection
Temperature:	Ambient	Ambient	Ambient
Detection:	UV 220 nm	UV 220/280 nm	UV 220/280 nm

Select Flow Rate Based on Column Diameter and Particle Size

2.1 mm id Columns		4.6 mm id Columns	
Particle Size, µm	**Flow Rate, mL/min**	**Particle Size, µm**	**Flow Rate, mL/min**
5	0.1-0.5	1.7	0.1-0.3
1.7	0.1-0.8	3	0.1-0.5
		5	0.1-0.8
		10	0.1-1.0

Optimize Conditions

Some separations may require a specific buffer, ionic strength, pH, and/or temperature

Ionic Strength:

Certain ionic strength is required to sustain the function of columns. Usually, a minimal concentration of 10-20 mM salt is required. However, greater than 20 mM strength may prevent the adsorption of biomolecules onto the column. Commonly used salts are sodium and potassium chloride and acetate. For elution, a typical salt concentration is 400-500 mM.

Note: Never use water alone for washing columns as it causes a significant increase in backpressure.

Selection of Buffers and pH:

Buffers play a key role in the optimization of separations. Phosphate buffers are typically used for antibodies and many biomolecules. The following are also recommended: MES, Tris, and ACES buffers. Use buffers of pH 5.0-6.5. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher pH (>pH 6.5) may be needed. Phosphoric acid, acetic acid, HCl and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Selection of Buffers and pH:

For anion-exchange, acetate and phosphate buffers of pH 8.0-9.0 are recommended. pH can be adjusted usually by +/- 0.2 units. For some specific proteins, buffers with higher or lower pH may be needed. Phosphoric acid, acetic acid, HCl and NaOH can be used to adjust pH.

pH gradients can also be used for elution.

Additives

Organic Solvents:

Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents:

Non-ionic, anionic, and zwitterionic detergents can be used. Cationic detergents are not recommended.

Additives

Organic Solvents:

Acetonitrile, ethanol, methanol, and other similar solvents can be used up to 50%.

Detergents:

Non-ionic, cationic, and zwitterionic detergents can be used. Anionic detergents are not recommended.

Temperature:

Agilent Bio MAb and IEX columns are stable up to 80 °C. However, many proteins and biomolecules are heat labile. Be sure to establish the temperature stability of your sample before routinely using high temperature for separation.

SEC Column Methods

Choose Initial Columns and Conditions for Size-Based Separation of Biomolecules, Aggregation Analysis – Peptides, Polypeptides, and Proteins

Peptides, Polypeptides, Proteins MW >0.1-1,250 kDa

Peptides, Polypeptides, Proteins MW >0.1-10,000 kDa

Select Column Based on Molecular Weight Range and Pore Size

Agilent Bio SEC-3 (3 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250

Agilent Bio SEC-5 (5 µm)

Pore Size	MW range, kDa
100Å	0.1-100
150Å	0.5-150
300Å	5-1,250
500Å	15-5,000
1000Å	50-7,500
2000Å	>10,000

Recommended Initial Separation Conditions

Column: Agilent Bio SEC (3 µm and 5 µm)

Mobile Phase: 150 mM phosphate buffer, pH 7.0*

Gradient: Isocratic in 30-60 min range

Temperature: Recommended: 10-30 °C, Maximum: 80 °C

Flow Rate: 0.1-0.4 mL/min for 4.6 mm id columns
0.1-1.25 mL/min for 7.8 mm id columns

Sample Size: ≤ 5% of total column volume

*Other aqueous buffers with high and low salt can be used

For additional information, see application note: *Defining the Optimum Parameters for Efficient Size Separations of Proteins* (publication # 5990-8895EN)
www.agilent.com/chem/library

After the initial chromatogram, additional changes may be needed to improve the separation, maintain protein solubility, or to decrease sample interaction with the chromatographic media. The ionic strength of the mobile phase can be adjusted up or down in strength to attain an optimized separation. pH can also be adjusted usually + 0.2 units. If further optimization is necessary, the upward or downward range should be expanded. A change of temperature or addition of an organic solvent can also be used.

For protocols requiring additional salt, these buffers are typical:

100-150 mM sodium chloride in 50 mM sodium phosphate, pH 7.0

100-150 mM sodium sulfate in 50 mM sodium phosphate, pH 7.0

50-100 mM urea in 50 mM sodium phosphate, pH 7.0

Other similar salts (e.g. KCl) and guanidine hydrochloride can also be used

pH Range:

2.0-8.5

Potential organic solvent additions include:

5-10% ethanol (or other similar solvents) in 50 mM sodium phosphate, pH 7.0

5% DMSO in 50 mM sodium phosphate, pH 7.0

Temperature:

Typically, SEC separations are run at 20-30 °C. Separation of proteins and peptides may require higher temperature to improve both resolution and recovery of proteins and hydrophobic peptides.

Maximum temperature of Bio SEC columns is 80 °C

High Sensitivity Capillary Column Methods

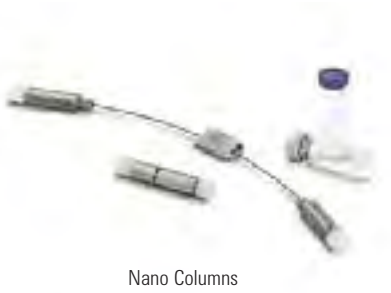
Mobile Phase Considerations

Low pH

TFA is generally not used for LC/MS separations of proteins and peptides. The first step is normally to replace TFA with 0.1 to 1% formic acid. Acetic acid, up to 1% can also be used as an alternative mobile phase modifier. At low pH, the best separation may still be obtained with TFA in the mobile phase. In some cases, the TFA can be displaced post-column with an alternative acid, such as propionic acid.

Mid and High pH

LC/MS can also be done at high pH with 10-20 mM NH_4OH as a mobile phase additive.



Nano Columns

Capillary and Nano Columns

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

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

TIPS & TOOLS

Agilent offers a variety of e-Seminars and on-site training to help you learn how to be a more effective chromatographer.

For more information, visit
www.agilent.com/chem/education

ZORBAX Nano columns for high sensitivity protein digest analysis by LC/MS

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

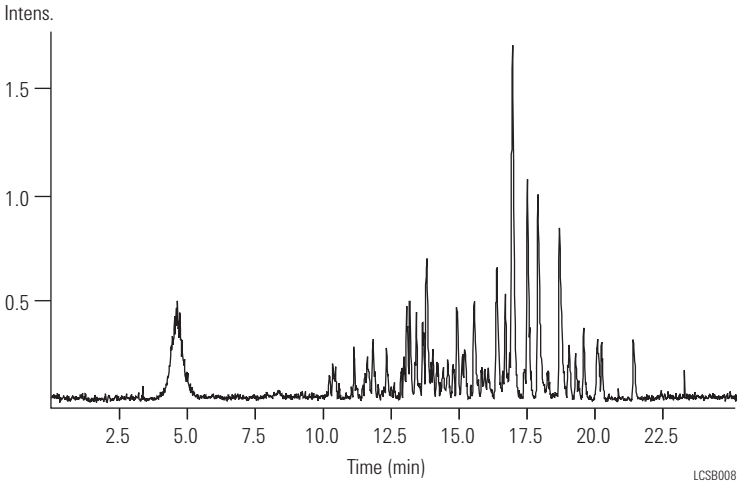
Mobile Phase: A: Water + 0.1% Formic acid,
B: ACN + 0.1% Formic acid

Flow Rate: 600 nL/min

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

Detector: Positive Ion Nano Electrospray MS

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



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

High sensitivity with capillary columns

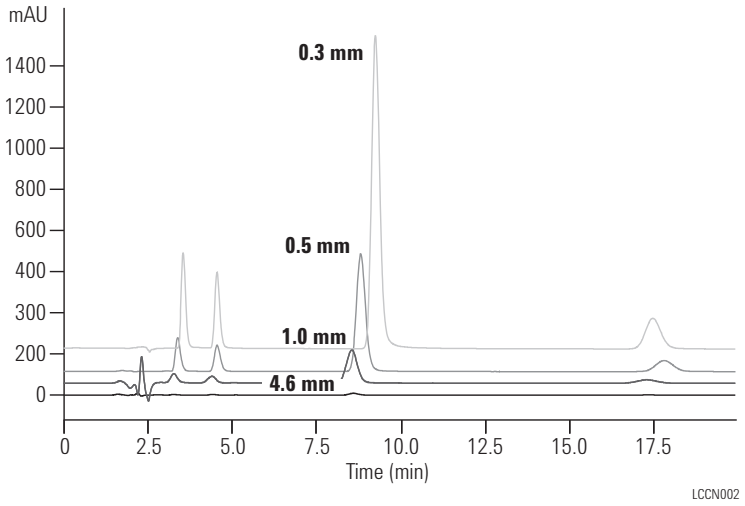
Column: **ZORBAX SB-C18**
5064-8255
0.3 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
5064-8256
0.5 x 150 mm, 5 µm

Column: **ZORBAX SB-C18**
863600-902
1.0 x 150 mm, 3.5 µm

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

Sample: 200 ng Biphenyl



Sample-limited applications require capillary column dimensions to minimize on-column sample dilution and to enhance sensitivity. The 0.3 mm capillary in this example provides 100 times more sensitivity than the standard 4.6 mm column. Agilent Nanobore (0.1 mm to 0.075 mm id) columns can provide up to 2,000 times more sensitivity for your most limited sample applications.

Human serum: Low abundance protein isolation and identification from 1-D gel band by LC/MS

Column: **ZORBAX 300SB-C18**
Trap: 0.3 x 5 mm, 5 µm, 5065-9913
Analytical: 0.3 x 150 mm, 5 µm, 5064-8263

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 6 µL/min

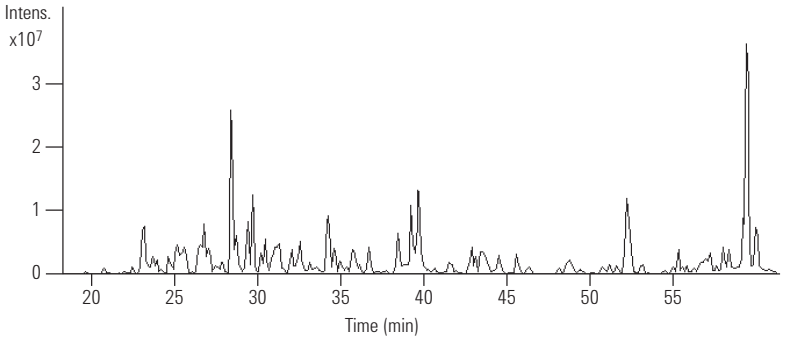
Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

Sample: Band from 1-D in gel digest

Proteins Identified

1. α-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

Base Peak Chromatogram



Sample Preparation of Human Serum:
Major serum proteins removed using Multiple Affinity Removal Column:
4.6 x 100 mm, P/N 5185-5985
Followed by 1-D gel digest

Peptide phosphorylation sites LC and LC/MS using Capillary LC columns

Column: **ZORBAX 300SB-C18**
5064-8268
0.5 x 150 mm, 3.5 µm

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

Flow Rate: 5.5 µL/min

Gradient: 5-55% B in 50 min, to
85% B from 55-57 min

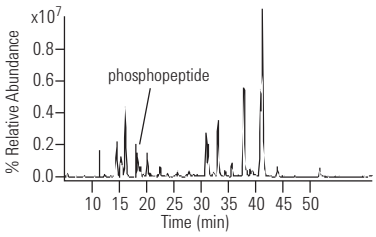
Detector: UV, 206 nm

MS Conditions:

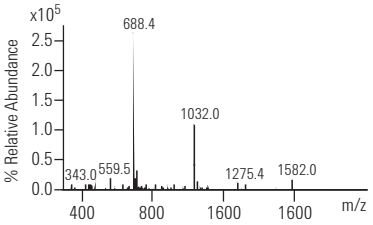
LC/MS:	Pos. Ion ESI with LC/MSD trap
Vcap:	4000 V
Drying gas flow:	7 L/min
Drying gas temperature:	250 °C
Nebulizer:	15 psi
Capillary Exit Volt:	50 V Max
Accum Time:	300 ms
Total Averages:	3
Isolation Width:	3 m/z
Frag Ampitude:	1.0 V

Sample: Beta case in digest, 100 nL (4 pmol)

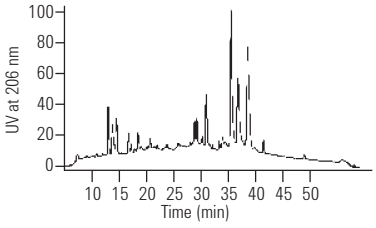
MS



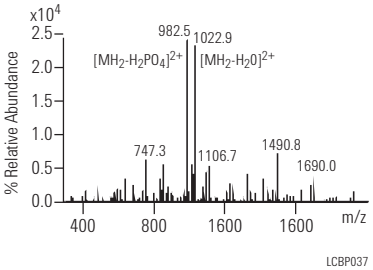
Full Scan MS



UV



MS/MS of [M+2H]$^{2+}$ at m/z 1032



Capillary columns for HPLC analyses with UV and MS detection

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

Mobile Phase: 5-55% B in 50 min, to 85% B from 55-57 min
A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

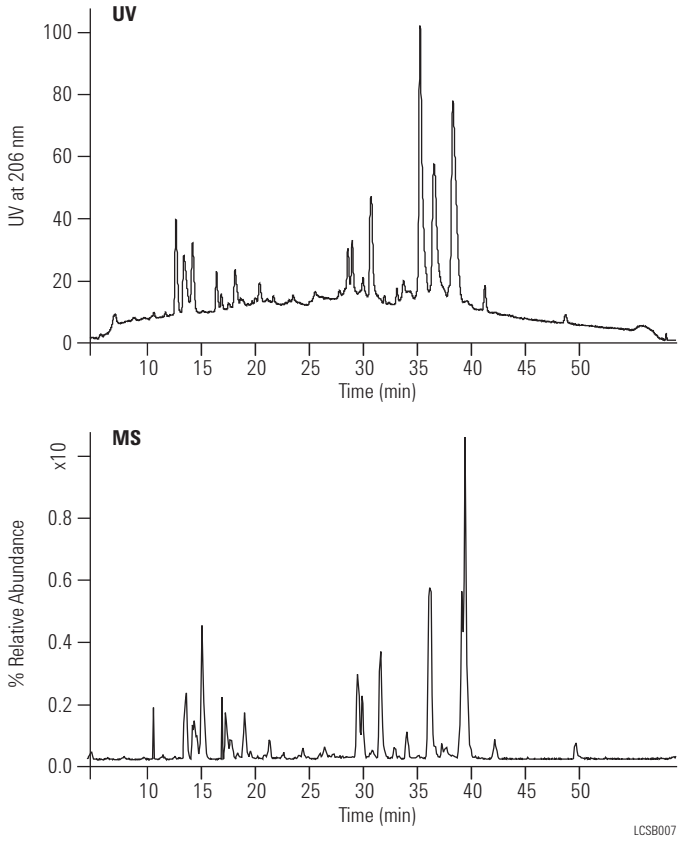
Flow Rate: 5.5 µL/min

Detector: UV, 206 nm

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

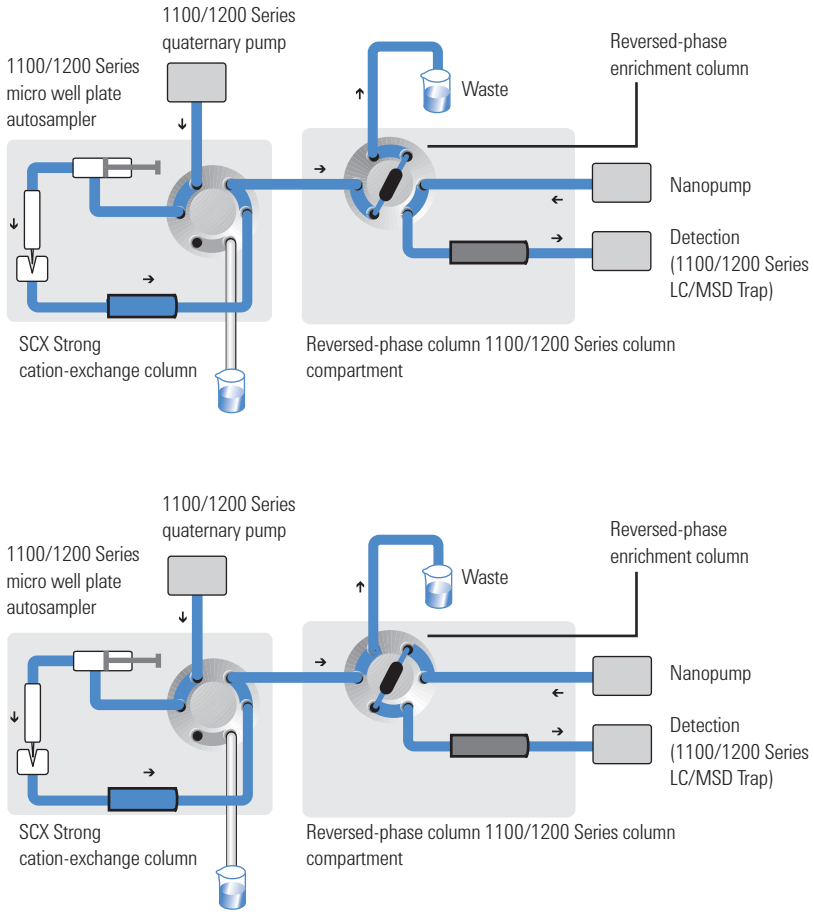
Sample: 100 nL
Beta Casein Digest (4 pmol)

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



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

Typical Column Configuration for 2-D HPLC



Flow path of an Agilent customized Nanoflow Proteomics Solution system.
1. Sample loading, elution from SCX and trapping on enrichment column
2. Valve switch in column compartment, elution from enrichment column; separation on RP, and MS anaylsis

Proteins in a complex sample by 2-D HPLC with Nano HPLC columns

Column: **ZORBAX 300SB-C18**
5065-9913
0.3 x 5 mm, 5 µm

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

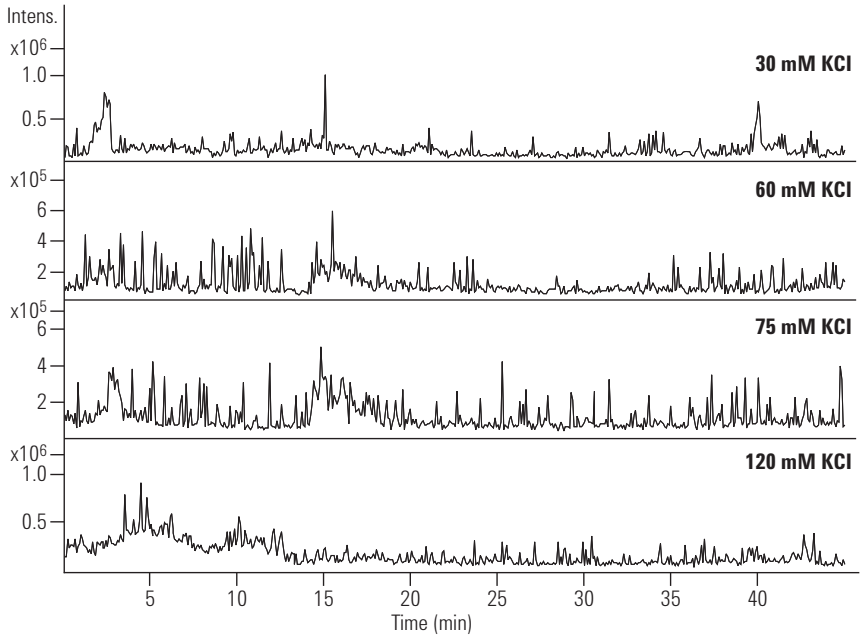
Mobile Phase: Quaternary Pump: 3% Acetonitrile/0.1% Formic acid
Nanopump: A = Water, 0.1% Formic acid, B = ACN, 0.1% Formic acid

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

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

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

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



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

Nano Columns

ZORBAX Bio-SCX Series II

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

Column Specifications

Bonded Phase	Pore Size	Surface Area	pH Range	Functionality	Max Pressure
ZORBAX Bio-SCX Series II	300Å	90 m^2/g	2.5-8.5	Sulfonic acid	350 bar

ZORBAX Bio-SCX Series II

Description	Size (mm)	Particle Size (µm)	Bio-SCX Series II
Capillary	0.3 x 35	3.5	5065-9912
Capillary	0.8 x 50	3.5	5065-9942

ZORBAX Bio-SCX Series II provides more retention of small peptides

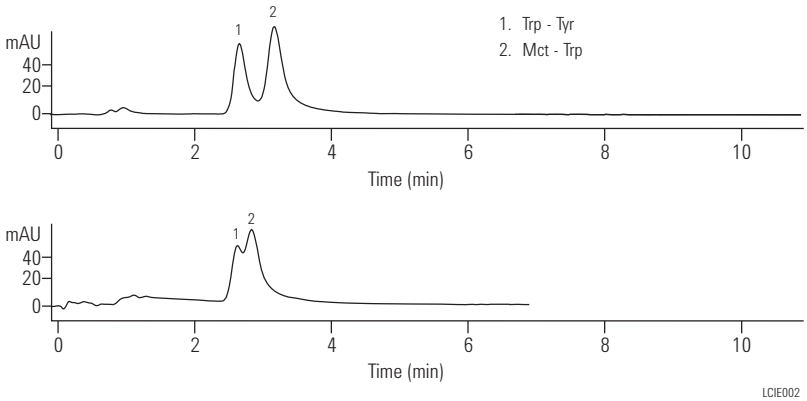
Column: **ZORBAX Bio SCX Series II 5065-9912 0.3 x 35 mm, 3.5 µm**

Mobile Phase: 95% 40 mM NaCl: 5% ACN, 0.3% Formic acid

Flow Rate: 5 µL/min

Detector: 230 nm

Sample: Synthetic Dipeptides



LCIE002

The new ZORBAX Bio-SCX Series II column retains smaller peptides more strongly than some other SCX columns. The result is increased resolution of more hydrophilic peptides fragments and more accurate identification when these columns are used in 2-D HPLC analysis.

ZORBAX HPLC Capillary Columns (glass-lined stainless steel)

Description	Size (mm)	Particle Size (µm)	300SB-C18	300SB-C8	Poroshell 300SB-C8	300Extend-C18	Bio-SCX Series II
Capillary	0.8 x 50	3.5					5065-9942
Capillary	0.5 x 250	5	5064-8266				
Capillary	0.5 x 150	5	5064-8264				
Capillary RR	0.5 x 150	3.5	5064-8268				
Capillary	0.5 x 75	5			5065-4468		
Capillary	0.5 x 35	5	5064-8294				
Capillary RR	0.5 x 35	3.5	5065-4459				
Capillary	0.3 x 250	5	5064-8265				
Capillary	0.3 x 150	5	5064-8263				
Capillary	0.3 x 35	5	5064-8295				
Capillary	0.3 x 35	3.5					5065-9912
Capillary RR	0.3 x 150	3.5	5064-8267	5065-4460		5065-4464	
Capillary RR	0.3 x 100	3.5	5064-8259	5065-4461		5065-4465	
Capillary RR	0.3 x 75	3.5	5064-8270	5065-4462		5065-4466	
Capillary RR	0.3 x 50	3.5	5064-8300	5065-4463		5065-4467	
Replacement Screens, 10/pk			5065-4427	5065-4427	5065-4427	5065-4427	

ZORBAX 300SB-C18 trap/guard, 5065-9913

ZORBAX Nano HPLC Columns (PEEK)

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

MicroBore (1.0 mm id) Columns

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

Agilent MicroBore (1.0 mm id) columns are a good choice when sample sizes are limited. They can improve detection limits 5 times over 2.1 mm id columns when the same sample mass is used. This increase in sensitivity can be critical. MicroBore columns use low flow rates (typically ~ 50 µL/min). Therefore, these columns are ideal for use with detectors requiring low flow rates such as some mass spectrometers and with capillary LC systems.

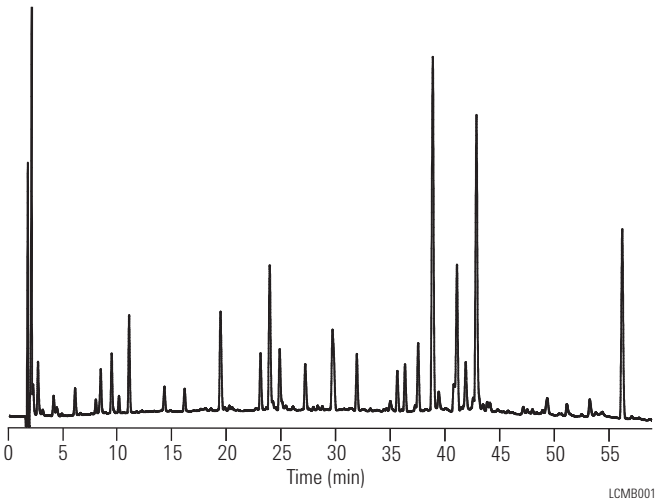
Optimum performance is achieved when MicroBore columns are used with UHPLC/HPLC Microbore systems. A wide variety of bonded phases is available for use up to 400 bar including StableBond, 300SB-C18, 300SB-C8, and Poroshell columns. Polymeric reversed-phase, PLRP-S, and ion-exchange PL-SAX and PL-SCX are also available for applications requiring exceptionally stable wide pore particles. Guard columns are also now available with an adjustable tube stop depth to provide a perfect zero dead volume connection every time.

Sterically Protected 300StableBond Bonded Phase

Separation of a tryptic digest on ZORBAX MicroBore 300SB-C18

Column:	**ZORBAX 300SB-C18** **863630-902** **1.0 x 150 mm, 3.5 µm**
Mobile Phase:	Gradient: 2-60% B in 60 min A: 0.1% TFA B: 0.075% TFA/80% ACN
Flow Rate:	50 µL/min
Temperature:	50 °C
Detector:	UV, 215 nm
Sample:	2 µL Tryptic Digest of rhGH

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



Microbore HPLC for sensitive peptide analysis

Column: **PLRP-S 100Å 5 µm, 150 mm x various id**

Mobile Phase: A: 0.01 M Tris HCl, pH 8
B: A + 0.35 M NaCl, pH 8

Flow Rate: 1 mL/min

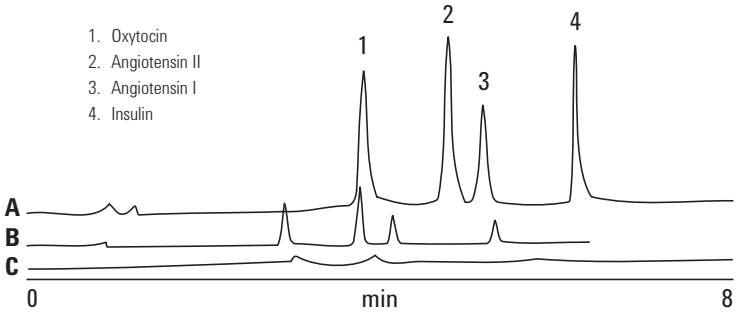
Gradient: Linear 20% ACN, 0.1% TFA to 50% ACN, 0.1% TFA over 15 min

Injection Volume: 0.5 µL

Sample Conc: 0.25 mg/mL

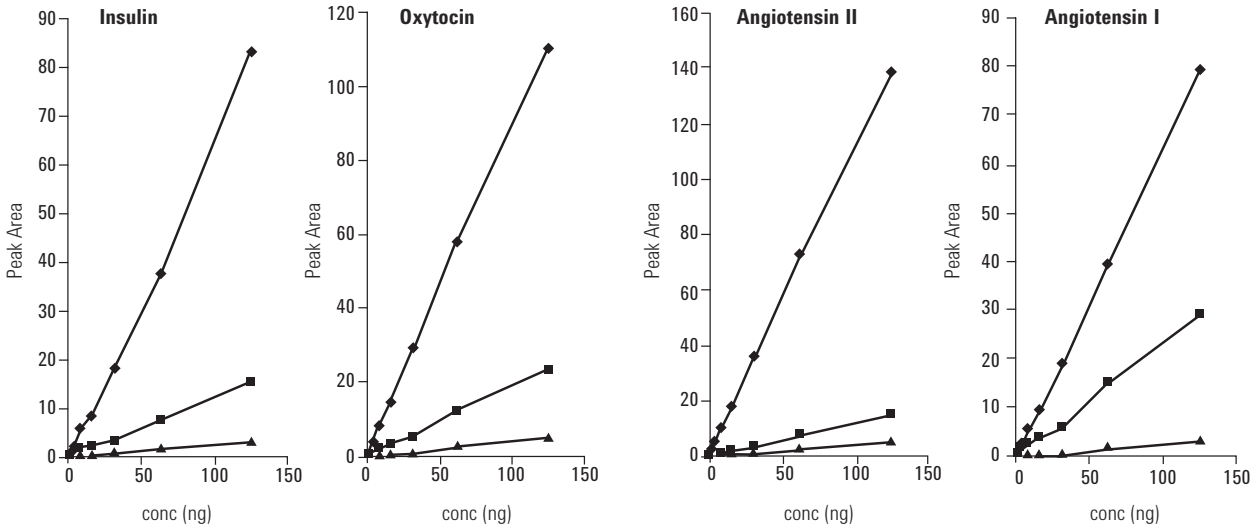
Detector: UV, 220 nm

Peak Identification
A. 1.0 mm id (flow rate 47 µL/min)
B. 2.1 mm id (flow rate 200 µL/min)
C. 4.6 mm id (flow rate 1 mL/min)



Peptide separation on Agilent PLRP-S 100Å 5 µm columns

Peak Identification
◆ 1.0 mm
■ 2.1 mm
▲ 4.6 mm



Standard curve data-point graphs on Agilent PLRP-S columns

MicroBore (1.0 mm id)

Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7		
MicroBore	1.0 x 250	5	861630-902			
MicroBore RR	1.0 x 150	3.5	863630-902	863630-906		
MicroBore RR	1.0 x 50	3.5	865630-902	865630-906		
MicroBore Guard, 3/pk	1.0 x 17	5	5185-5920	5185-5920		

Description	Size (mm)	Particle Size (µm)	Poroshell 300SB-C18	Poroshell 300SB-C8	Poroshell 300SB-C3	Poroshell 300Extend-C18
MicroBore	1.0 x 75	5	661750-902	661750-906	661750-909	671750-902
MicroBore Guard, 3/pk	1.0 x 17	5	5185-5968	5185-5968	5185-5968	

Description	Size (mm)	Particle Size (µm)	PLRP-S 100Å USP L21	PLRP-S 300Å USP L21	PLRP-S 1000Å USP L21	PLRP-S 4000Å USP L21
MicroBore	1.0 x 150	3	PL1312-3300			
MicroBore	1.0 x 50	3	PL1312-1300	PL1312-1301		
MicroBore	1.0 x 50	5	PL1312-1500	PL1312-1501	PL1312-1502	PL1312-1503
MicroBore	1.0 x 50	8			PL1312-1802	PL1312-1803

Description	Size (mm)	Particle Size (µm)	PL-SCX 1000Å	PL-SCX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
MicroBore	1.0 x 50	5	PL1351-1502	PL1351-1503	PL1345-1502	PL1345-1503

Polymeric Prep HPLC Columns

Purification – Prep HPLC

Agilent has a comprehensive range of silica and polymeric HPLC columns and media designed for biomolecule purification. There are high efficiency small particle prep columns optimized for the purification of µg and mg amounts of a biopharmaceutical drug candidate and fully porous bulk media, to pack development and process columns to purify multiple 100 g, kg and multi-kg of API.

Some columns are specifically designed to address the needs of high efficiency purification, while other products provide easy scale-up from small particle analytical columns to full scale API production. **Table 1** shows prep column/media options and the quantity of product that can be purified.

BioPharmaceutical Lifecycle		Discovery	Development	Production
		µg mg	g	kg multi-kg
		high efficiency		high throughput
Reversed-Phase	**mRP-C18**	→		
	ZORBAX Prep HT 300Å StableBond	→	→	
	VariTide RPC	→	→	
	PLRP-S 100Å, 300Å, 1000Å, 4000Å	→	→	→
	PL-SAX	→	→	→
Ion-Exchange	**PL-SCX**	→	→	→
Size Exclusion	**ZORBAX GF-250/450**	→	→	

Table 1: Agilent columns and media for biomolecule purification – chromatographic type, product family and purification scale.

Purification Column Selection

Application	Technique	Notes	Agilent Columns
Proteomics	Reversed-Phase	A specialist high recovery column for proteomics applications. It is designed for µg scale purifications with maximum recovery.	mRP-C18
All Biomolecules	Reversed-Phase	High efficiency 300Å silica-based particles.	ZORBAX PrepHT 300SB
Synthetic Peptides	Reversed-Phase	Polymeric material designed for the purification of synthetic peptides. It is a high efficiency single-column solution for the full range of synthetic peptides, acidic, basic, hydrophobic and hydrophilic, and covers the size range of peptides produced by both solution and solid phase synthesis.	VariTide RPC
All Biomolecules	Reversed-Phase	The premium polymeric reversed-phase family with a range of pore sizes and particle sizes to enable high efficiency laboratory scale purification using small particle prep column, and scale-up to high yield production purification with larger particles at the process scale. Use PLRP-S when purification will be scaled up to produce APIs and will need regulatory documentation.	PLRP-S
		• 3 µm and 5 µm for high efficiency • 8 µm, 10 µm, 10-15 µm, 15-20 µm, 30 µm and 50 µm particles for larger scale and low pressure purification	
All Biomolecules	Ion-Exchange	A fully porous strong anion-exchanger	PL-SAX
		• 5 µm particle size for high efficiency separations • 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification	
		A fully porous strong cation-exchanger	PL-SCX
		• 5 µm particle size for high efficiency separations • 8 µm, 10 µm and 30 µm particles for larger scale medium and low pressure purification	

TIPS & TOOLS

Further information can be found in the following publication:

Biomolecule Purification
(publication # 5990-8335EN)

www.agilent.com/chem/library

ZORBAX 300Å StableBond Prep HT Cartridge Columns

ZORBAX PrepHT

High purity, high recovery, and high throughput can be easily achieved with Agilent ZORBAX PrepHT columns. These are available in a variety of bonded phases – StableBond 300Å, C18, C8, C3, and CN – for optimized resolution and loadability under any conditions.

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

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

ZORBAX 300Å StableBond

Hardware	Description	Size (mm)	Particle Size (µm)	300SB-C18 USP L1	300SB-C8 USP L7	300SB-CN USP L10	300SB-C3 USP L56
PrepHT Cartridge Columns (require endfittings kit 820400-901)							
	PrepHT Cartridge	21.2 x 250	7	897250-102	897250-106	897250-105	897250-109
	PrepHT Cartridge	21.2 x 150	7	897150-102	897150-106		897150-109
	PrepHT Cartridge	21.2 x 150	5	895150-902	895150-906		895150-909
	PrepHT Cartridge	21.2 x 100	5	895100-902	895100-906		895100-909
	PrepHT Cartridge	21.2 x 50	5	895050-902	895050-906		895050-909
	PrepHT Endfittings, 2/pk			820400-901	820400-901	820400-901	820400-901
	PrepHT Guard Cartridge, 2/pk	17.0 x 7.5	5	820212-921	820212-918	820212-924	820212-924
	Guard Cartridge Hardware			820444-901	820444-901	820444-901	820444-901

PLRP-S for Prep to Process

- Discovery stage to multi-kg cGMP production reduces method development time
- Chemical stability for separations, optimization, sanitation, and regeneration increases selectivity and column lifetime
- Single batch packing of multiple columns reduces system downtime and validation costs

The PLRP-S media, rigid poly(styrene/divinylbenzene) particles, are available in a range of pore sizes for small molecule, synthetic biomolecule and macromolecule purification. Their thermal and chemical stability makes them ideal for purifications that require extreme conditions for sample preparation, compound elution, and column regeneration.

Capacity and resolution are two key parameters for maximizing the throughput of a purification. With a wide choice of pore sizes and extended range of operating conditions, PLRP-S provides more options to achieve the optimum process. Particle sizes range from 3 µm to 50 µm for scale-up from the µg/mg discovery stage to multi-kg cGMP production. Excellent chemical stability, up to 1 M NaOH, permits sanitation and regeneration that increase column lifetime. PLRP-S media batch sizes of up to 600 L are available, providing single batch packing of multiple columns.

As part of our commitment to quality and continuity of supply, all manufacturing is carried out under a fully documented process. A Type II Drug Master File and regulatory support files are available for process materials, and facility audits are routinely conducted.

PLRP-S Prep to Process Application Guide

Application	PLRP-S Media Pore Size			
	100Å	300Å	1000Å	4000Å
Synthetic biomolecules, peptides, and oligonucleotides	✓	✓		
Recombinant biomolecules, peptides, and proteins	✓	✓		
Large biomolecules, antibodies, DNA fragments			✓	✓
Small molecules, unstable compounds including metal sensitivity	✓			

Column Specifications

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

Purification of a 25-mer trityl-off oligonucleotide and analytical quantitation of the fraction using PLRP-S 100Å, 4.6 x 50 mm

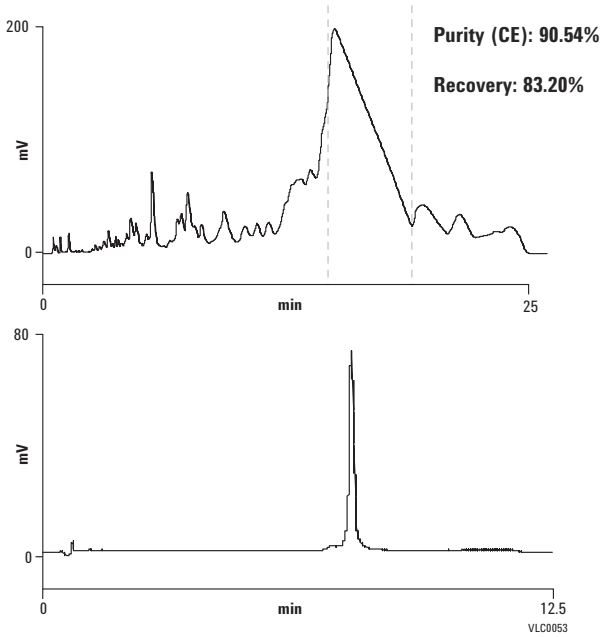
Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

Mobile Phase: A: 100 mM Triethylammonium acetate (TEAA)
B: 100 mM TEAA in 25:75 Acetonitrile:water

Flow Rate: 1 mL/min

Gradient: 25% B 0 min, 35% B 2 min, 45% B 22.5 min, 45% B 23 min, 25% B 23.05 min, 25% B 26 min

Temperature: 80 °C



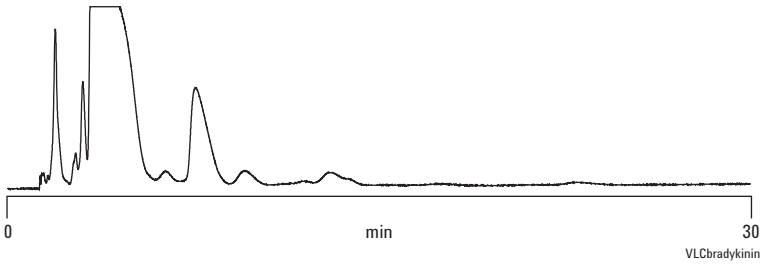
Crude bradykinin prep load

Column: **PLRP-S 100Å**
PL1512-5100
4.6 x 250 mm, 10 µm

Sample: 30 µL containing 1.5 mg of crude peptide

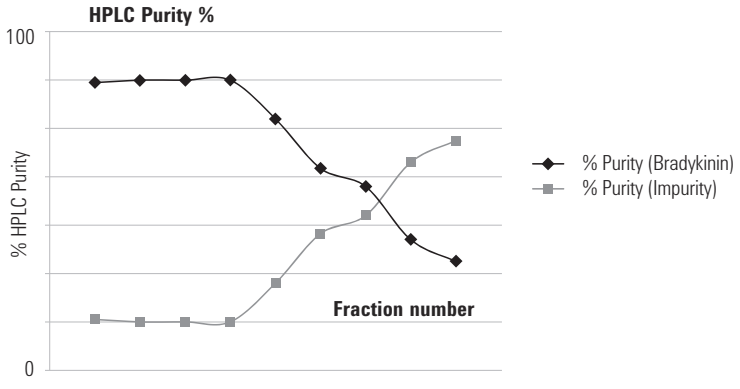
Mobile Phase: 0.1% TFA in 21% ACN:79% water

Flow Rate: 1 mL/min (360 cm/hr)



Fraction analysis – the concentration overload purification

HPLC analysis of the fractions collected across the peak showed that fractions 1 to 4 contained only the peptide of interest and that the level of the critical impurity increased with increasing fraction number. Using the high efficiency PLRP-S column it was possible to obtain from the crude, 91.7% pure, a recovery of 97% with 100% purity. For more information, see application note 5990-7736EN.



Prep to Process PLRP-S

Size (mm)	Particle Size (µm)	PLRP-S 100Å	PLRP-S 300Å	PLRP-S 1000Å	PLRP-S 4000Å
100 x 300	30			PL1812-3102	PL1812-3103
100 x 300	15-20	PL1812-6200	PL1812-6201		
100 x 300	10-15	PL1812-6400	PL1812-6401		
100 x 300	10	PL1812-6100	PL1812-6101		
100 x 300	8	PL1812-6800	PL1812-6801		
50 x 300	8	PL1712-6800	PL1712-6801		
50 x 150	30			PL1712-3702	PL1712-3703
50 x 150	15-20	PL1712-3200	PL1712-3201		
50 x 150	10-15	PL1712-3400	PL1712-3401		
50 x 150	10	PL1712-3100	PL1712-3101	PL1712-3102	PL1712-3103
50 x 150	8	PL1712-3800	PL1712-3801		
25 x 300	15-20	PL1212-6200	PL1212-6201		
25 x 300	10-15	PL1212-6400	PL1212-6401		
25 x 300	10	PL1212-6100	PL1212-6101		
25 x 300	8	PL1212-6800	PL1212-6801		
25 x 150	30			PL1212-3702	PL1212-3703
25 x 150	10	PL1212-3100	PL1212-3101	PL1712-3102	PL1712-3103
25 x 150	8	PL1212-3800	PL1212-3801		
25 x 50	10			PL1212-1102	PL1212-1103
PLRP-S Method Development Columns					
4.6 x 250	30			PL1512-5702	PL1512-5703
4.6 x 250	15-20	PL1512-5200	PL1512-5201		
4.6 x 250	10-15	PL1512-5400	PL1512-5401		
4.6 x 250	10	PL1512-5100	PL1512-5101	PL1512-5102	PL1512-5103
4.6 x 250	8	PL1512-5800	PL1512-5801		
4.6 x 150	30			PL1512-3702	PL1512-3703
4.6 x 150	15-20	PL1512-3200	PL1512-3201		
4.6 x 150	10-15		PL1512-3401		
4.6 x 150	10	PL1512-3100	PL1512-3101	PL1512-3102	PL1512-3103
4.6 x 150	8	PL1512-3800	PL1512-3801		

PLRP-S Bulk Media

Particle Size (µm)	Unit	PLRP-S 100Å	PLRP-S 300Å	PLRP-S 1000Å	PLRP-S 4000Å
50	1 kg	PL1412-6K00	PL1412-6K01	PL1412-6K02	
	100 g	PL1412-4K00	PL1412-4K01	PL1412-4K02	
30	1 kg			PL1412-6702	PL1412-6703
	100 g			PL1412-4702	PL1412-4703
15-20	1 kg	PL1412-6200	PL1412-6201		
	100 g	PL1412-4200	PL1412-4201		
10-15	1 kg	PL1412-6400	PL1412-6401		
	100 g	PL1412-4400	PL1412-4401		
10	1 kg	PL1412-6100	PL1412-6101	PL1412-6102	PL1412-6103
	100 g	PL1412-4100	PL1412-4101	PL1412-4102	PL1412-4103
8	1 kg	PL1412-6800	PL1412-6801		

For larger quantities, please contact your local Agilent sales office

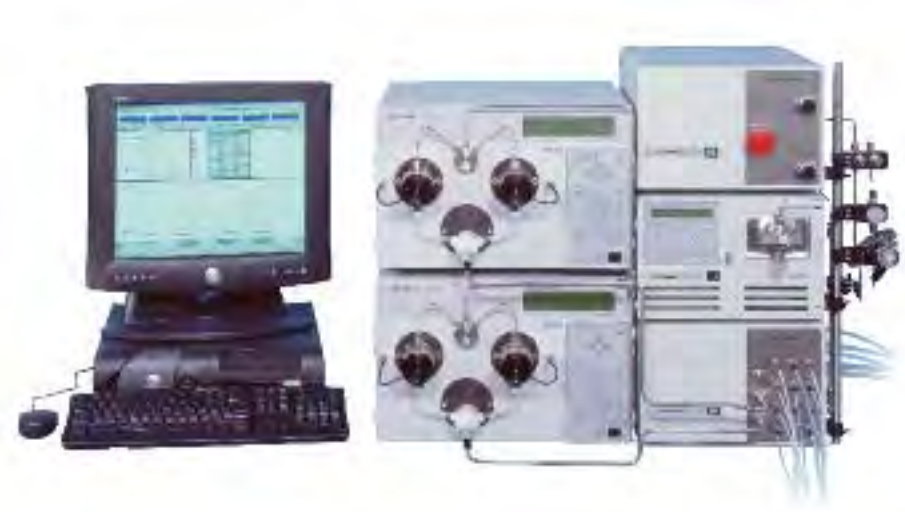
PL-SAX and PL-SCX for Prep to Process

- Ion-exchange purifications over a wider pH range extend applications
- HPLC flow rates and rapid equilibration reduce purification cycle times
- Large pore size for improved mass transfer delivers high speed, high resolution purifications

These rigid, strong ion-exchange materials are extremely hydrophilic and are designed for purification of biomolecules. The PL-SAX and PL-SCX materials are totally polymeric and are chemically and thermally stable over a full range of HPLC conditions. The strong ion-exchange functionalities, covalently linked to a chemically stable polymer, facilitate ion-exchange purifications over a wider pH range. This stability can be exploited for column sanitation and clean-up. Thermal stability also enables the use of denaturing conditions and stabilizing/solubilizing agents for the purification of target compounds, as encountered in the purification of synthetic oligonucleotides with self-complementary sequences.

Both the 1000Å and 4000Å wide-pore materials are mechanically stable and robust and can be operated over a wide range of linear velocities, with fast loading of dilute solutions and wash cycles. HPLC flow rates and rapid equilibration reduces purification cycle times.

Packing in dynamic axial compression (DAC) column hardware is straightforward and high efficiency columns are achieved with excellent reproducibility and lifetimes. The 1000Å pore size is for high-capacity purifications and the 4000Å gigaporous particles with improved mass transfer are intended for large biomolecules and high-speed, high-resolution purifications.



Column Specifications		
	PL-SAX	**PL-SCX**
Matrix	Fully polymeric	Fully polymeric
Pore Sizes	1000Å, 4000Å	1000Å, 4000Å
Particle Sizes	10 µm, 30 µm	10 µm, 30 µm
Bead Form	Rigid spherical	Rigid spherical
Functionality	Quaternary amine	Sulfonic acid
Pressure Stability	3000 psi	3000 psi
Temperature Stability	80 °C	80 °C
pH Range	1-14	1-14
Eluent Compatibility	All anion-exchange buffers	All cation-exchange buffers
Packed Bed Density	0.39 g/mL	0.39 g/mL

Purification of a large oligonucleotide

Column: **PL-SAX 1000Å, 8 µm**

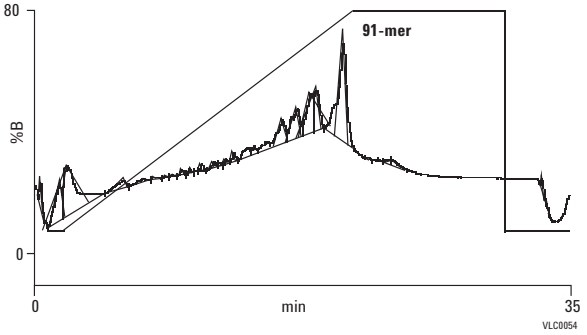
Mobile Phase: A: 93% 0.1 M TEAA, pH 7:7% ACN
B: 93% 0.1 M TEAA, 3.24 M ammonium acetate, pH 7:7% ACN

Gradient: 0-100% B in 20 min

Flow Rate: 1.5 mL/min

Temperature: 60 °C

Detector: UV, 290 nm



Preparative fractionation of a culture filtrate containing amyloglucosidases on Agilent PL-SAX 4000Å

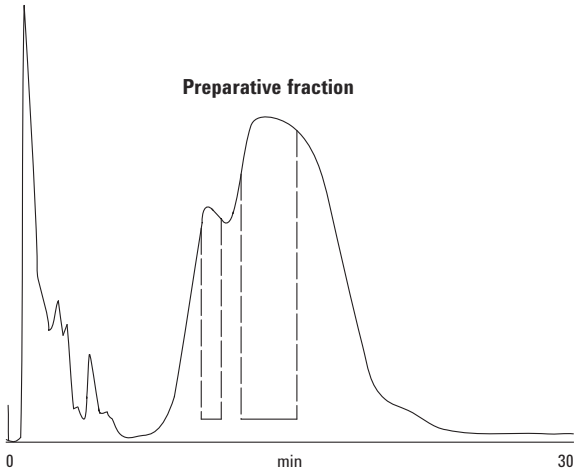
Column: **PL-SAX**
PL1551-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.01 M Tris HCl, pH 8
B: A + 0.5 M NaCl, pH 8

Flow Rate: 4.0 mL/min

Gradient: Linear 0-100% B in 2 min

Detector: UV, 280 nm



Prep to Process PL-SAX and PL-SCX Columns and Bulk Media

Prep to Process PL-SAX and PL-SCX

Dimensions	Particle Size (µm)	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
100 x 300	30	PL1851-3102	PL1851-3103	PL1845-3102	PL1845-3103
100 x 300	10	PL1851-2102	PL1851-2103	PL1845-2102	PL1845-2103
50 x 150	30	PL1751-3702	PL1751-3703	PL1745-3702	PL1745-3703
50 x 150	10	PL1751-3102	PL1751-3103	PL1745-3102	PL1745-3103
25 x 150	30	PL1251-3702	PL1251-3703	PL1245-3702	PL1245-3703
25 x 150	10	PL1251-3102	PL1251-3103	PL1245-3102	PL1245-3103
25 x 50	10	PL1251-1102	PL1251-1103	PL1245-1102	PL1245-1103
7.5 x 150	8	PL1151-3802	PL1151-3803		
7.5 x 50	8	PL1151-1802	PL1151-1803	PL1145-1802	PL1145-1803
PL-SAX and PL-SCX Method Development Columns					
4.6 x 250	30	PL1551-5702	PL1551-5703	PL1545-5702	PL1545-5703
4.6 x 250	10	PL1551-5102	PL1551-5103	PL1545-5102	PL1545-5103
4.6 x 150	30	PL1551-3702	PL1551-3703	PL1545-3702	PL1545-3703
4.6 x 150	10	PL1551-3102	PL1551-3103	PL1545-3102	PL1545-3103

PL-SAX and PL-SCX Bulk Media

Particle Size (µm)	Unit	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
30	1 kg	PL1451-6702	PL1451-6703	PL1445-6702	PL1445-6703
	100 g	PL1451-4702	PL1451-4703	PL1445-4702	PL1445-4703
10	1 kg	PL1451-6102	PL1451-6103	PL1445-6102	PL1445-6103
	100 g	PL1451-4102	PL1451-4103	PL1445-4102	PL1445-4103

For larger quantities, please contact your local Agilent sales office

Peptide Purification

VariTide RPC Columns

VariTide is a cost-effective solution for the production of synthetic peptides. This column lets you manage the cost and efficiency of high-volume synthetic peptide purification, from µg to g scale. VariTide provides a solution for peptide houses that manufacture small quantities of hundreds or thousands of peptides where manufacturing time is the economic driving force.

VariTide RPC Columns for Synthetic Peptides

- A single column to cover the full range of synthetic peptides
- Small particle size for maximum efficiency, even with 1 and 2 in prep columns
- Bulk media to pack 1 and 2 in prep columns for the purification of mg to g quantities

VariTide RPC columns and media are part of the VariPep Peptide Solution. This is the recommended option for cost-effective separation and purification of synthetic peptides using generic methods.

VariTide RPC Columns for Synthetic Peptides

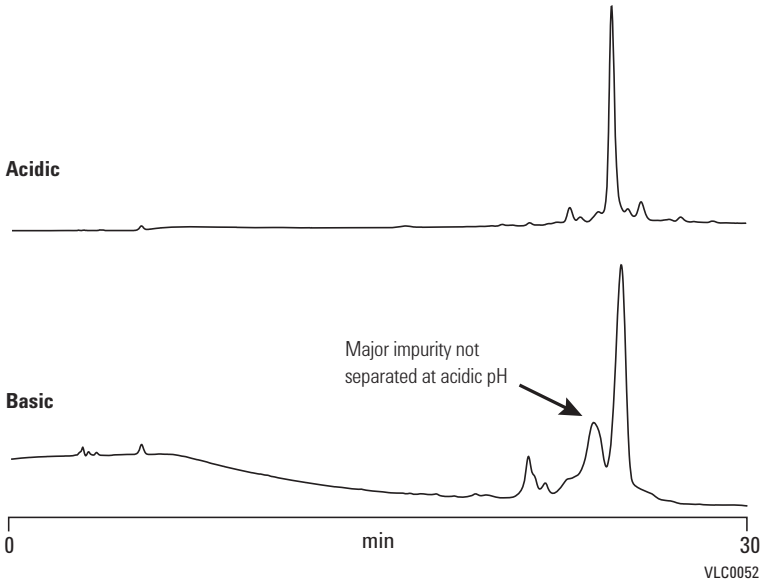
Size (mm)	Part No.
21.2 x 250	PL1E12-5A05
10.0 x 250	PL1012-5A05
4.6 x 250	PL1512-5A05

VariTide RPC Bulk Media

Description	Part No.
100 g	PL1412-4A05
1 kg	PL1412-6A05

Crude peptide screen

Column:	**VariTide RPC PL1512-5A05 4.6 x 250**
Mobile Phase:	Acidic A: 0.1% TFA in 95% water: 5% ACN B: 0.1% TFA in 50% water: 50% ACN Basic A: 5% ACN, 95% 20 mm ammonium carbonate pH 9.5 B: 50% ACN, 50% 20 mm ammonium carbonate pH 9.5
Flow Rate:	1.0 mL/min (360 cm/h)
Gradient:	0-100% B in 30 min
Detector:	UV, 220 nm



VariPure IPE

- Pre-packed for convenience
- Removal of ion-pairing agents for improved productivity
- High performance and economy for excellent efficiency

VariPure IPE is a polymer-supported quaternary-amine resin with a bicarbonate counter ion, designed for removing acidic ion-pair reagents, such as trifluoroacetic acid (TFA), formic acid or acetic acid. VariPure IPE is a high performance and economical acid removal material conveniently supplied as pre-packed SPE type devices. The particle size, capacity and device geometry are matched to provide sufficient residence time to achieve effective ion-air extraction under gravity flow. For acid labile peptides, removal of the ion-pairing agent prevents acid degradation of the peptide during post-HPLC work-up, and increases the yield of purified product.

VariPure IPE

Loading	Counter-ion Removal Capacity	Unit	Part No.
100 mg per 3 mL tube	~ 5 mL 0.1% TFA	50/pk	PL3540-D603VP
500 mg per 6 mL tube	~ 25 mL 0.1% TFA	50/pk	PL3540-C603VP
1 g per 20 mL tube	~ 50 mL 0.1% TFA	25/pk	PL3540-P603VP
25 g			PL3549-3603VP

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Rapid Analysis of Adenovirus Type 5 Particles with Bio-Monolith Anion-Exchange HPLC Columns to Support the Development of a High-Titre Manufacturing Platform	Bio-Monolith QA	Adenovirus	5990-5524EN	Application Note
Separation of Two Sulfurated Amino Acids with other Seventeen Amino Acids by HPLC with Pre-Column Derivatization	Eclipse Plus-C18	Amino acid analysis	5990-5977EN	Application Note
Rapid, Accurate, Sensitive, and Reproducible HPLC Analysis of Amino Acids	ZORBAX Eclipse AAA	Amino acid analysis	5980-1193EN	Application Note
High-Speed Amino Acid Analysis (AAA) on 1.8 µm Reversed-Phase (RP) Columns	ZORBAX Eclipse Plus	Amino acid analysis	5989-6297EN	Application Note
Improved Amino Acid Methods Using Agilent ZORBAX Eclipse Plus C18 Columns for a Variety of Agilent LC Instrumentation and Separation Goals	ZORBAX Eclipse Plus	Amino acid analysis	5990-4547EN	Application Note
Rapid and Precise Determination of Cellular Amino Acid Flux Rates using HPLC with Automated Derivatization with Absorbance Detection	ZORBAX Eclipse Plus	Amino acid analysis	5990-3283EN	Application Note
Agilent PL-SAX 1000Å HPLC Columns and Media	PL-SAX	Analysis/Prep - Oligonucleotides	5990-8200EN	Flyer
Compliance for Biopharmaceutical Laboratories	LC columns	Compliance	5990-7001EN	Primer
Macroporous Reversed-Phase C18 High-Recovery Protein Fractionation HPLC Column	mRP-C18	Human serum, Biomarkers	5989-2714EN	Brochure
Rapid Human Polyclonal IgG Quantification using the Agilent Bio-Monolith Protein A HPLC Column	Bio-Monolith	IgG	5989-9733EN	Application Note
Rapid IgM Quantification in Cell Culture Production and Purification Process Monitoring using the Agilent Bio-Monolith QA Column	Bio-Monolith QA	IgM	5989-9674EN	Application Note
Optimization of Protein Separations on Weak Cation-Exchange Columns – a Study of the Particle Size, Buffer Salts and Gradients	Bio IEX	MAbs	5990-8833EN	Technical Poster

(Continued)

BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
pH Gradient Elution for Improved Separation of Monoclonal Antibody Charged Variants	Bio MAb	MAbs	5990-9629EN	Application Note
Characterization of Monoclonal Antibodies on the Agilent 1260 Infinity Bio-inert Quaternary LC by Size Exclusion Chromatography using the Agilent Bio SEC Columns	Bio SEC	MAbs	5990-6414EN	Application Note
Agilent BioHPLC Columns for the Characterization of Monoclonal Antibodies	Biocolumns	MAbs	5990-7753EN	Flyer
Fast Separation of Monoclonal Antibody and Dimer by SEC with Agilent Bio SEC	Bio SEC	MAbs	5990-8613EN	Application Note
Choosing a ZORBAX Poroshell Phase (C3, C8, or C18) for Fast Separation of Monoclonal Antibodies	Poroshell 300	MAbs	5989-0071EN	Application Note
Determination of the Glycosylation Status of Intact Recombinant Human Antibodies using Time of Flight Mass Spectrometry	Poroshell 300	MAbs	N/A	Technical Poster
High Speed and Ultra-High Speed Peptide Mapping of Human Monoclonal IgG on Poroshell 300SB-C18, C8, and C3	Poroshell 300	MAbs	5989-0590EN	Application Note
Rapid HPLC Analysis of Monoclonal Antibody IgG1 Heavy Chains using ZORBAX Poroshell 300SB-C8	Poroshell 300	MAbs	5989-0070EN	Application Note
Comparison of ZORBAX StableBond 300Å LC Columns to Optimize Selectivity for Antibody Separations Using HPLC and LC/MS	ZORBAX 300SB	MAbs	5989-6840EN	Application Note
Ultra High Speed and High Resolution Separations of Reduced and Intact Monoclonal Antibodies with Agilent ZORBAX RRHD Sub-2 µm 300 Diphenyl UHPLC Column	ZORBAX RRHD 300-Diphenyl	MAbs	5990-9668EN	Application Note
Reversed-Phase Optimization for Ultra Fast Profiling of Intact and Reduced Monoclonal Antibodies using Agilent ZORBAX Rapid Resolution High Definition 300SB-C3 Column	ZORBAX RRHD 300SB-C3	MAbs	5990-9667EN	Application Note

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BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Reversed-Phase Separation of Intact Monoclonal Antibodies (MAb) using Agilent ZORBAX RRHD 300SB-C8	ZORBAX RRHD 300SB-C8	MAbs	5990-9016EN	Application Note
Rapid UHPLC Analysis of Reduced Monoclonal Antibodies using an Agilent ZORBAX Rapid Resolution High Definition (RRHD) 300SB-C8 Column	ZORBAX RRHD 300SB-C8	MAbs	5990-9631EN	Application Note
Increased UV-Sensitivity in Combination with Novel WCX Column Separation for Better Detectability of Charge State Variants of Biotherapeutic Proteins	Bio MAb	MAbs and other proteins	N/A	Technical Poster
Agilent HPLC Column Selection Guide	HPLC columns	Many	5990-4435EN	Selection Guide
The LC Handbook: Guide to LC Columns and Method Development	LC columns	Method development	5990-7595EN	Primer
Agilent PLRP-S 100Å HPLC Columns and Media	PLRP-S	Oligonucleotides	5990-8187EN	Flyer
HPLC Purification of 26-bp Serial Analysis of Gene Expression Ditags	PLRP-S	Oligonucleotides	5990-7739EN	Application Note
Improved Column Lifetime with Thermally Stable Polymer Columns for Oligonucleotide Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7764EN	Application Note
Ion-Pair Reversed-Phase Purification of De-Protected Oligonucleotides – Choice of Pore Size	PLRP-S	Oligonucleotides	5990-7763EN	Application Note
Use Temperature to Enhance Oligonucleotide Mass Transfer and Improve Resolution in Ion-Pair RP HPLC	PLRP-S	Oligonucleotides	5990-7765EN	Application Note
High Resolution Separations of Oligonucleotides using PL-SAX Strong Anion-Exchange HPLC Columns	PL-SAX	Oligonucleotides	5990-8297EN	Application Note
Fast Impurity Profiling of Synthetic Oligonucleotides with the Agilent 1290 Infinity LC System and Agilent 6530 Accurate-Mass QTOF LC/MS	ZORBAX Eclipse Plus C18 RRHD	Oligonucleotides	5990-5825EN	Application Note
Agilent PLRP-S Media and Load & Lock Columns – The Future of Prep/Process Chromatography	Prep/Process	Oligonucleotides, Peptides, Proteins	5990-8201EN	Flyer
Agilent PLRP-S 50 µm HPLC Media	PLRP-S	Oligonucleotides, Peptides, Small proteins	5990-8188EN	Flyer

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BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Analysis of Peptides on a PLRP-S 100Å 10 µm with ELS Detection and Acetonitrile-Free Eluents	PLRP-S	Peptides	5990-7760EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides	PLRP-S	Peptides	5990-7740EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a SepTech ST150 10-C18	SepTech	Peptides	5990-7951EN	Application Note
Investigation into the Alternatives to Acetonitrile for the Analysis of Peptides on a VariTide RPC	VariTide RPC	Peptides	5990-8145EN	Application Note
Fast Monitoring of Bacteriophage Production During Fermentation Using the Agilent Bio-Monolith HPLC Column	Bio-Monolith	Phage production, process monitoring	5990-3247EN	Application Note
Physicochemical Characterization of a Therapeutic Protein by Peptide Mapping, SEC and IEX using the Agilent 1260 Infinity Bio-inert Quaternary LC System	Bio MAb, Bio SEC, ZORBAX Eclipse Plus, Poroshell 120	Protein analysis	5990-6192EN	Application Note
Optimization of the Agilent 1100 HPLC System for Superior Results with ZORBAX Poroshell Columns	Poroshell 300	Protein analysis	5988-9998EN	Application Note
Using Poroshell 300SB-C18 for High-Sensitivity, High-Throughput Protein Analysis on the Agilent LC/MSD	Poroshell 300-C18	Protein analysis	5988-7031EN	Application Note
Analysis of Albumin Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7852EN	Application Note
Analysis of Complex Bacterial Cell Division Proteins by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8143EN	Application Note
Analysis of Globulins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7851EN	Application Note
Analysis of Hsp47, a Collagen Chaperone, by Size Exclusion Chromatography (SEC)	ProSEC 300S	Protein analysis	5990-8142EN	Application Note
Analysis of Various Globular Proteins using ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7850EN	Application Note
Effect of pH on Protein Size Exclusion Chromatography	ProSEC 300S	Protein analysis	5990-8138EN	Application Note
Globular Proteins and the Calibration of ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7767EN	Application Note

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BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Reduce Tubing Volume to Optimize Column Performance	Small diameter columns	Optimizing instrument performance	5990-4964EN	Application Note
Using the High-pH Stability of ZORBAX Poroshell 300Extend-C18 to Increase Signal-to-Noise in LC/MS	ZORBAX 300 Extend-C18	Optimizing instrument performance	5989-0683EN	Application Note
Increase Sensitivity with Microbore Polymeric HPLC Columns from Agilent	PLRP-S (Microbore)	Peptide hormone, small proteins, small molecules	5990-8666EN	Technical Overview
Decreasing Analysis Time Using Poroshell 300SB-C18 in Analysis of a Protein Digest	Poroshell 300	Peptide mapping	5988-6081EN	Application Note
Rapid Peptide Mapping Method with High Resolution using a sub 2-µm Column	ZORBAX 300SB-C18	Peptide mapping	5990-4712EN	Application Note
Increased Peak Capacity for Peptide Analysis with the Agilent 1290 Infinity LC System	ZORBAX Eclipse Plus	Peptide mapping	5990-6313EN	Application Note
Trypsin-Digested Monoclonal Antibody and BSA using Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Peptide mapping	5990-8244EN	Application Note
Preparative Scale Purification of Bradykinin by Concentration Overload	PLRP-S	Peptide purification	5990-7736EN	Application Note
Preparative Scale Purification of Bradykinin by Volume Overload	PLRP-S	Peptide purification	5990-7741EN	Application Note
Preparative Scale Purification of Depherelin by Concentration Overload	PLRP-S	Peptide purification	5990-7742EN	Application Note
Preparative Scale Purification of Leuprolide by Concentration Overload	PLRP-S	Peptide purification	5990-7735EN	Application Note
Superior Resolution of Peptides on SepTech ST150 10-C18 using Acetonitrile-Free Gradient Elution	SepTech	Peptide purification	5990-7761EN	Application Note
Agilent PLRP-S Media for HPLC Analysis of Peptides	PLRP-S	Peptides	5990-8667EN	Technical Overview

(Continued)

BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Light Scattering Analysis of BSA with ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7766EN	Application Note
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Protein analysis	5990-7939EN	Application Note
LC Handbook and Compliance Guide to Recombinant Protein Characterization	N/A	Protein analysis	5990-8561EN	Primer
Agilent ZORBAX 300SB-C18 1.8µm Rapid Resolution High Definition Columns for Proteins	ZORBAX 300SB-C18	Protein analysis	5990-7989EN	Technical Overview
Analysis of Oxidized Insulin Chains using Reversed-Phase Agilent ZORBAX RRHD 300SB-C18	ZORBAX RRHD 300SB-C18	Protein analysis	5990-7988EN	Application Note
Fast Separation of Recombinant Human Erythropoietin using Reversed-Phase Agilent ZORBAX RRHD 300SB-C18, 1.8 µm	ZORBAX RRHD 300SB-C18	Protein analysis	5990-9248EN	Application Note
ACN-free HPLC Analysis and Prep Purification of ACP Fragment	PLRP-S	Protein purification	5990-7762EN	Application Note
Isocratic Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Protein purification	5990-7737EN	Application Note
Agilent PL-SAX Anion-Exchange Media for Amyloglucosidase Purification and Analysis	PL-SAX	Protein purification	5990-8664EN	Technical Overview
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Protein purification	5990-8141EN	Application Note
Optimizing Protein Separations with Agilent Weak Cation-Exchange Columns	Bio IEX	Protein separation	5990-9628EN	Application Note
Faster Separations Using Agilent Weak Cation-Exchange Columns	Bio IEX	Protein separation	5990-9931EN	Application Note
Optimum Pore Size for Characterizing Biomolecules with Agilent Bio SEC Columns	Bio SEC	Protein separation	5990-9894EN	Application Note
Separation of High MW Fibrous Proteins	PLRP-S	Protein separation	5990-8137EN	Application Note

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BioHPLC Columns Literature				
Title	**Column/Product**	**Application**	**Publication Number**	**Publication Type**
Fast Protein Separations Using Agilent Poroshell 300	Poroshell 300	Protein separation	5989-9899EN	Application Note
Fast Separation of Large and Heterogeneous Proteins using ZORBAX Poroshell C18, C8, and C3 Phases	Poroshell 300	Protein separation	5989-0015EN	Application Note
Protein Identification and Impurity Profiling using Wide-Pore Reversed-Phase HPLC/UHPLC	Poroshell 300	Protein separation	5991-0625EN	Brochure
Use of Temperature to Increase Resolution in the Ultrafast HPLC Separation of Proteins with ZORBAX Poroshell 300SB-C8 HPLC Columns	Poroshell 300-C8	Protein separation	5989-0589EN	Application Note
The Effect of NaCl Concentration on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8139EN	Application Note
The Effect of Temperature on Protein Size Exclusion Chromatography	ProSEC 300S	Protein separation	5990-8140EN	Application Note
Infinitely Better for Bio-Molecule Analysis	Agilent 1260 Infinity Bio-inert Quaternary LC System	Proteins	5990-6220EN	Brochure
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8832EN	Technical Poster
Defining the Optimum Parameters for Efficient Size Separations of Proteins	Bio SEC	Proteins	5990-8895EN	Application Note
Compliance for Biopharmaceutical Laboratories	Many	Proteins	5990-7001EN	Primer
Gradient Purification of Synthetic Acyl Carrier Protein Fragment 65-74	PLRP-S	Proteins	5990-7738EN	Application Note
Fast Agilent HPLC for Large Biomolecules	PLRP-S, PL-SAX, PL-SCX	Proteins	5990-8663EN	Technical Overview
Agilent Anion-Exchange Media for Proteins – Loading vs Resolution – Effect of Flow Rate and Example Protein Separations	PL-SAX	Proteins	5990-8777EN	Technical Overview
Purity Assessment Following Affinity Separation	PL-SAX	Proteins	5990-8436EN	Technical Overview
Agilent PL-SCX Cation-Exchange Media for Large Biomolecules	PL-SCX	Proteins	5990-8665EN	Technical Overview

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BioHPLC Columns Literature

Title	Column/Product	Application	Publication Number	Publication Type
Poroshell 300SB-C18 for Fast, High Protein Separation	Poroshell 300	Proteins	5988-2100ENUS	Brochure
Progressive Denaturation of Globular Proteins in Urea	ProSEC 300S	Proteins	5990-8141EN	Application Note
ProSEC 300S Columns Protein Characterization Columns	ProSEC 300S	Proteins	5990-7468EN	Flyer
Static Light Scattering Analysis of Globular Proteins with Agilent ProSEC 300S Columns	ProSEC 300S	Proteins	5990-7939EN	Application Note
Confidently Separate and Characterize Biomolecules with Agilent BioHPLC Columns	Bio SEC, Bio IEX, Bio MAb	Proteins	5990-5195EN	Brochure
Increase your Productivity with Agilent ZORBAX RRHD 300Å 1.8 µm Columns	ZORBAX RRHD 300SB-C18, C8	Proteins, Peptides	5990-8124EN	Flyer
High Purity, High Recovery, High Throughput – Agilent Technologies Offers Two New Lines of Preparative HPLC Columns	Agilent Prep HT	Purification/Prep	5989-2350EN	Brochure
Biomolecule Purification – Purification Columns and Media for Peptides, Oligonucleotides, and Proteins	PLRP-S, PL-SAX, PL-SCX	Purification/Prep	5990-8335EN	Brochure
The Influence of Silica Pore Size on Efficiency, Resolution and Loading in Reversed-Phase HPLC	SepTech	Purification/Prep	5990-8298EN	Application Note
Analysis of Protein Primary Structure when using Wide-Pore sub-2-µm Particles and UHPLC	ZORBAX RRHD 300SB-C18	Purification/Prep	5990-8830EN	Technical Poster
Polyethylene Glycol/Oxide Standards and the Calibration of Agilent ProSEC 300S Columns	ProSEC 300S	SEC	5990-8147EN	Application Note

TIPS & TOOLS

For the latest application notes and new product information, go to **www.agilent.com/chem/library**

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GPC/SEC Columns and Standards

- A full portfolio of products for analysis of synthetic and natural polymers
- A wide selection of polymer standards to cover the range of applications in organic and water based solvents
- PL aquagel-OH-series, for aqueous SEC separations, and PLgel, for organic polymer applications, are available in mixed and individual pore sizes across a range of particle sizes, to cover the full spectrum of molecular weights (MW)
- Prep scale columns are available, along with narrow bore columns and columns designed for specific applications

Gel permeation chromatography (GPC) and size exclusion chromatography (SEC) are names applied to the most popular technique for measuring the molecular weight distribution (MWD) of natural and synthetic polymers, a property that affects many of the physical parameters of materials such as strength, toughness and chemical resistance. GPC and SEC are liquid chromatographic techniques that separate individual polymer chains on the basis of their size in solution and not on their chemistry. Gel permeation chromatography (GPC) is the name used to describe the analysis of polymers in organic solvents, such as tetrahydrofuran. Size exclusion chromatography (SEC) is the name used to describe the analysis of polymers in water and water-based solvents, such as buffer solutions. GPC/SEC is the only established method for obtaining a comprehensive understanding of a polymer's molecular weight distribution.

TIPS & TOOLS

For information on SEC columns for proteins, turn to pages 416-417.

Table of Contents

LC and LC/MS Columns for GPC/SEC

GPC/SEC Columns

The key to successful GPC/SEC separations is the correct choice of columns. The comprehensive range of Agilent products for GPC/SEC has been designed to cover virtually all polymer analysis application areas, and to make selection for the correct column, solvent, and calibration standard fast and reliable.

Agilent's PLgel GPC series of columns are for polymer applications using organic solvents. PLgel is a highly cross-linked, porous polystyrene/divinylbenzene matrix, which is recognized as a market leader in GPC column technology. PLgel materials have high pore volume and high-efficiency to maximize resolution. Their unequalled solvent compatability makes for easy transfer between polar and non-polar eluents, and outstanding physical rigidity provides extended lifetimes that maximize downtime. For more information and full ordering details, see pages 496-497.

Agilent's PL aquagel-OH series of columns provide a chemically and physically stable matrix for reliable aqueous SEC separations. The columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality. The "neutral" surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of compounds with neutral, ionic, and hydrophobic moieties, alone or in combination. PL aquagel-OH is available for analytical and preparative applications. For more information and full ordering details, see page 523.

Polymer standards for GPC/SEC

Agilent manufactures the highest quality polymer standards with extremely narrow polydispersity and the widest molecular weight range commercially available. These quality polymer standards are supplied with extensive characterization data utilizing a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign the peak molecular weight (Mp).

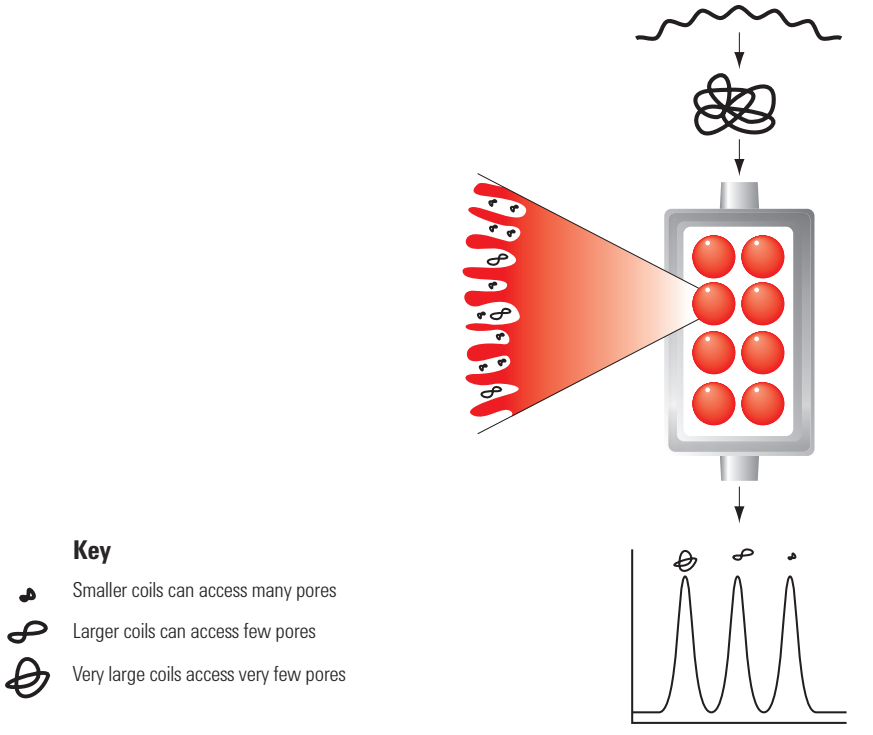
EasiVial – for organic and aqueous calibration. EasiVial is the fastest and most convenient method to deliver an accurate 12-point column calibration. EasiVial eliminates tedious weight procedures for improved calibration accuracy and reduces solvent dispensing to limit risks associated with handling solvents.

EasiCal – for organic solvents. EasiCal packs are pre-prepared for a no-fuss process. Two different combs, each with ten detachable spatulas, support a mixture of five polymer standards. The cost-effective format is designed to save money.

Individual standards and kits – an extensive range of polymer standard kits of different chemistries designed to match specific column sets are available, as well as individual standards in various pack sizes. For more details about Agilent's calibration standards for GPC/SEC, see page 530.

How GPC/SEC works:

- Polymer molecules dissolve in solution to form spherical coils with size dependent on molecular weight
- The polymer coils are introduced to eluent flowing through the column
- Columns are packed with insoluble porous beads with well-defined pore structure
- The size of pores is similar to that of the polymer coils
- The polymer coils diffuse in and out of the pores
- Result is elution based on size – large coils first, smaller coils last
- Size separation converted to molecular weight separation by use of a calibration curve constructed by the use of polymer standards



Mechanisms of GPC and SEC

Recommendations for setting up a GPC/SEC system

The following questions will help you find the recommended columns and standards for any given application, as well as system parameters such as injection volumes.

Choosing an eluent for GPC/SEC

Question	Answer	Recommendation	Comments
1. What is the sample soluble in?	Water or water buffer with up to 50% methanol	Agilent PL aquagel-OH	Best choice for water-based applications but cannot accommodate organics apart from methanol up to 50%
Many polymers are only soluble in a small number of solvents. This is the key question when developing methods for analyzing polymers. The solvents mentioned here are all common eluents employed in GPC/SEC.	Typical organic solvent such as THF, chloroform, toluene	Agilent PLgel or Agilent PlusPore	PLgel are the workhorse columns, PlusPore columns are an alternative
	Organic/water mixtures or polar organics such as, DMF, NMP	Agilent PolarGel	PolarGel is a smaller column range than PLgel or PL aquagel-OH columns but is suited to mixtures of organics and water

TIPS & TOOLS

More information on GPC/SEC instrumentation and systems is a click away. We have a variety of application notes, data sheets and brochures available from Agilent for free.

To learn more, visit
www.agilent.com/chem/gpc

Choosing a column for GPC/SEC

Columns shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
2. What is the expected molecular weight? *It may seem strange to ask this question, but in GPC/SEC the resolution of a column is related to the resolving range. Knowing something of the expected molecular weight of a sample helps to choose the best column that will give optimum results.*	High (up to several millions)	Aqueous solvents **PL aquagel-OH MIXED-H 8 µm** or combination of PL aquagel-OH 40 and 60 15 µm	The 15 µm column combination is best only where sample viscosity is very high, otherwise 8 µm columns give greater resolution
		Organic solvents **PLgel 10 µm MIXED-B** or PLgel 20 µm MIXED-A	The PLgel MIXED-A column resolves higher than the PLgel MIXED-B but at lower efficiency due to larger particle size
		Mixed solvents **PolarGel**	No PolarGel column available for this molecular weight range. Contact your local GPC/SEC expert for advice
	Intermediate (up to hundreds of thousands)	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	A wide-ranging column that covers most water-soluble polymers
		Organic solvents **PLgel 5 µm MIXED-C** or PLgel 5 µm MIXED-D, PolyPore or ResiPore	The PLgel columns are the most widely applicable for the majority of applications; PolyPore and ResiPore columns are alternatives
		Mixed solvents **PolarGel-M**	Covers most applications
	Low (up to tens of thousands)	Aqueous solvents **Combination of PL aquagel-OH 40 and PL aquagel-OH 30 8 µm**	These two columns in a combined set cover the low end of the molecular weight range
		Organic solvents **PLgel 3 µm MIXED-E** or MesoPore	The PLgel column provides high resolution and is designed for low molecular weight applications; the MesoPore column is an alternative
		Mixed solvents **PolarGel-L**	For low molecular weight applications
	Very low (a few thousand)	Aqueous solvents **PL aquagel-OH 20 5 µm**	This high-performance column gives high resolution at low molecular weight
		Organic solvents **OligoPore** or PLgel 3 µm 100Å	The OligoPore column is less prone to dispersion than the PLgel column, but both work well
		Mixed solvents **PLgel**	No PolarGel column covers this range so use PLgel columns as alternatives
	Unknown	Aqueous solvents **PL aquagel-OH MIXED-M 8 µm**	Covers the molecular weight ranges of most polymer samples
		Organic solvents **PLgel 5 µm MIXED-C** or PolyPore	This PLgel column is the most widely applicable for the majority of applications
		Mixed solvents **PolarGel-M**	Covers the majority of applications

Setting up the GPC/SEC system

Question	Answer	Recommendation	Comments
3. How many columns to use? *The greater the particle size of the media in the column (which is dependent on the expected molecular weight of the samples), the lower the resolution and the more columns are required to maintain the quality of the results. For higher molecular weight samples, larger particles are necessary to reduce the danger of shear degradation of samples during analysis.*	Depends on the particle size of the columns	Particle size 20 µm use 4 columns Particle size 13 µm use 3 columns Particle size 10 µm use 3 columns Particle size 8 µm use 2 columns Particle size 5 µm use 2 columns Particle size 3 µm use 2 columns	Increased number of columns required for large particle sizes to make up for low efficiencies

Question	Answer	Recommendation	Comments
4. What size injection volume? *The injection volume required is dependent on the particle size of the column – smaller particles need lower injection volumes to minimize dead volume. Larger injection volumes allow the introduction of high molecular weight samples at lower concentrations, reducing viscosity and ensuring a quality chromatogram is obtained.*	Depends on the particle size of the columns	Particle size 20 µm use 200 µL injection Particle size 13 µm use 200 µL injection Particle size 10 µm use 200 µL injection Particle size 5 µm use 100 to 200 µL injection Particle size 3 µm use 20 µL injection	Smaller particle sizes require smaller loops to minimize band broadening

What standards should I use?

Standards shown in bold are the best initial choice

Question	Answer	Recommendation	Comments
5. What is the eluent? *Standards are polymers, so the choice of standard mainly reflects solubility in the chosen eluents.*	Water or water buffer with up to 50% methanol	**Polyethylene glycol (PEG)/oxide (PEO)** or polysaccharides (SAC)	These standards perform in all water-based systems, PEG/PEO in convenient Agilent EasiVial format
	Typical organic solvent such as THF, chloroform, toluene	**Polystyrene (PS)** or polymethylmethacrylate (PMMA)	Polystyrene is the most commonly used standard in convenient EasiVial format
	Organic/water mixtures or polar organics such as DMF, NMP	**Polyethylene glycol/oxide** or polymethylmethacrylate	Polar standards perform well

(Continued)

What standards should I use?

Question	Answer	Recommendation	Comments
6. What format of standards are recommended?	For the quickest and simplest approach where accurate concentrations are not required	Easiest option – **EasiVial** or EasiCal	Simple to use, EasiVial preferred before EasiCal because of the wider choice of polymer types
Different formats of standards are available depending on customer preference.	If accurate concentrations are required	Accurate concentrations required – **EasiVial** or individual standards	Both formats allow accurate sample concentrations, EasiVials are simpler to use

Typical polymer molecular weights

If you are unsure of the molecular weight of your sample, the table below shows some approximate molecular weight ranges for common polymers, which will help you select the right column for your application.

Polymer Type	Typical molecular weight of polymer	Typical polydispersity[1] of polymer
Polymers from free radical synthesis	High (up to several million) Intermediate (up to hundreds of thousands)	~ 2
Polymers from ionic synthesis	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 1.01
Polymers from addition synthesis	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 2
Polymers from controlled radical polymerization	Low (up to tens of thousands) Very low (a few thousand)	~ 1.1 to 1.5
Polyolefins	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 200
Acrylates	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2
Small molecule additives	Very low (a few thousand)	1
Pre-polymers	Low (up to tens of thousands) Very low (a few thousand)	~ 2 to 10
Resins	Low (up to tens of thousands) Very low (a few thousand)	~ 2 to 10
Natural biopolymers such as polysaccharides	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 10
Rubbers	Intermediate (up to hundreds of thousands) High (up to several million)	~ 2 to 10
Biodegradable polymers	Intermediate (up to hundreds of thousands) Low (up to tens of thousands)	~ 1.1 to 2

[1] Polydispersity is a measure of the distribution of molecular mass of a polymer. Polydispersity index (PDI) = M_w/M_n.

Organic GPC

PLgel GPC Columns

- Robust performance under the most exacting conditions
- Temperature stability up to 220 °C
- Solvent compatibility allows easy and rapid transfer between solvents of varying polarity

PLgel materials have high pore volume and high efficiency to maximize resolution. Their unequalled solvent compatibility makes for easy transfer between polar and non-polar eluents, and outstanding physical rigidity provides extended lifetimes that minimize downtime.

The key to successful GPC separations is the correct choice of columns. The comprehensive range of PLgel products has been designed to cover virtually all organic solvent-based polymer analysis application areas, and to make selection of the correct column, solvent, and calibration standard fast and reliable.

PLgel is a highly cross-linked, porous polystyrene/divinylbenzene matrix, which is recognized as a market leader in GPC column technology. PLgel is manufactured to ISO 9001:2000 and benefits from comprehensive QC/QA for total reproducibility, batch-to-batch and column-to-column.

Solvent Compatibility

PLgel columns are routinely supplied in ethyl benzene* but you can easily and rapidly transfer between solvents of varying polarity. In organic GPC, sample to column interaction may occur occasionally and eluent modification can be used to eliminate these effects. PLgel columns are the ideal choice for such analyses, as they easily tolerate eluents in the pH range 1-14, as well as up to 10% water in a miscible organic solvent.

PLgel is compatible with all of these solvents

Solvent Polarity	Solvent
6.0	Perfluoroalkane
7.3	Hexane
8.2	Cyclohexane
8.9	Toluene
9.1	Ethyl acetate
9.1	Tetrahydrofuran (THF)
9.3	Chloroform
9.3	Methyl ethyl ketone (MEK)
9.7	Dichloromethane
9.8	Dichloroethene
9.9	Acetone
10.0	o-Dichlorobenzene (o-DCB)
10.0	Trichlorobenzene (TCB)
10.2	m-Cresol
10.2	o-Chlorophenol (o-CP)
10.7	Pyridine
10.8	Dimethyl acetamide (DMAc)
11.3	n-Methyl pyrolidone (NMP)
12.0	Dimethyl sulfoxide (DMSO)
12.1	Dimethyl formamide (DMF)

*We also provide a custom packing service in which columns can be shipped in specific solvents to provide extra convenience to our customers.

PLgel Frit Porosity

Media Type	Porosity (µm)
PLgel 3 µm	2
PLgel 5 µm	2
PLgel 10 µm	5
PLgel 20 µm	10

For PLgel column accessories ordering information please see page 529

PLgel MIXED Columns

The PLgel MIXED range greatly simplifies column selection for easy decision making. By using these mixed columns, you can eliminate mismatched column sets and spurious peaks for more reliable results. Every column contains a mixture of individual pore size materials, accurately blended to cover a specified broad range of molecular weight with a linear calibration to eliminate column mismatch. Simply add extra columns for even greater resolution.

Column Specifications

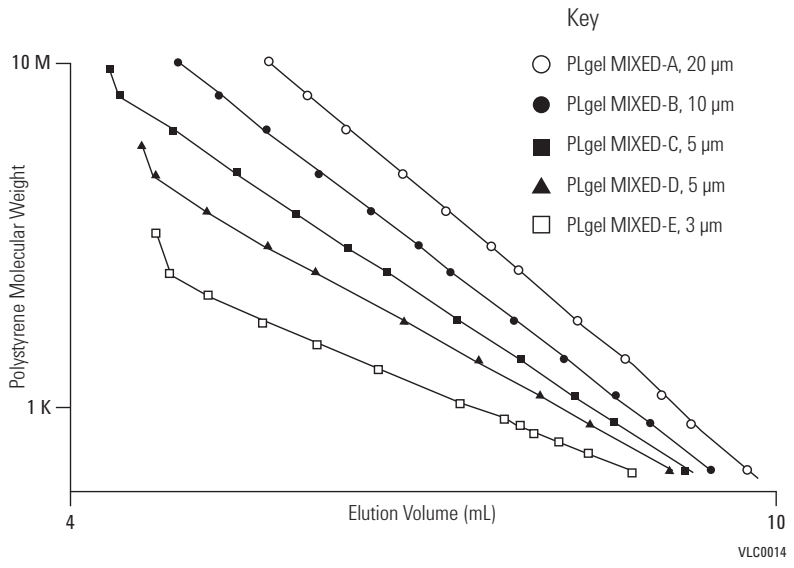
Column	Linear MW Operating Range (g/mol)	Guaranteed Column Efficiency	Typical Pressure	Maximum Flow Rate	Maximum Pressure	Maximum Temperature
PLgel MIXED-A	2,000-40,000,000	> 17,000 p/m	1 mL/min (7.5 mm id): ≈ 3 bar (44 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 2.4 bar (35 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	220 °C
PLgel MIXED-B	500-10,000,000	> 35,000 p/m	1 mL/min (7.5 mm id): ≈ 10 bar (145 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 8 bar (116 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	220 °C
PLgel MIXED-C	200-2,000,000	> 50,000 p/m	1 mL/min (7.5 mm id): ≈ 30 bar (435 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 24 bar (348 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	150 °C
PLgel MIXED-D	200-400,000	> 50,000 p/m	1 mL/min (7.5 mm id): ≈ 30 bar (435 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 24 bar (348 psi) per 250 mm (THF @ 20 °C, TCB @ 140 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	150 bar (2175 psi)	150 °C
PLgel MIXED-E	up to 30,000	7.5 x 300 mm: > 80,000 p/m 4.6 x 250 mm: > 70,000 p/m	1 mL/min (7.5 mm id): ≈ 50 bar (725 psi) per 300 mm 0.3 mL/min (4.6 mm id): ≈ 42 bar (609 psi) per 250 mm (THF @ 20 °C)	7.5 mm id: 1.5 mL/min 4.6 mm id: 0.5 mL/min	180 bar (2611 psi)	110 °C

PLgel MIXED Column Selection Guide

UHMW polymer distributions	PLgel MIXED-A, 20 µm
High MW polymers, demanding eluents	PLgel MIXED-B, 10 µm
Mid range MW polymers, high resolution	PLgel MIXED-C, 5 µm
Resins, condensation polymers	PLgel MIXED-D, 5 µm
Low MW resins, prepolymers	PLgel MIXED-E, 3 µm

PLgel MIXED Gel Calibration Curves

MIXED gel calibration curves are designed to be linear over a specified molecular weight range, ensuring that the same degree of resolution is achieved across the full operating range of the column. The particle size of the packing and porosity of a particular MIXED gel column are carefully matched to the MW range and application, thus optimizing performance and eliminating the effects of shear degradation. Resolution in GPC is controlled by the slope of the calibration curve and the particle size of the packing material. Agilent has scientifically determined the minimum number of MIXED gel columns required to perform accurate MWD determinations based on specific resolution (Rsp). Thus you can have complete confidence in the accuracy and precision of the calculated data.



Reference: Meehan, E. (1998) Size exclusion chromatography columns from Polymer Laboratories. In: Chi-San Wu (Ed.) Column Handbook for Size Exclusion Chromatography. Academic Press, New York, USA.

PLgel MIXED Columns

Description	Size (mm)	Part No.
PLgel 20 µm MIXED-A	7.5 x 300	PL1110-6200
PLgel 10 µm MIXED-B	7.5 x 300	PL1110-6100
PLgel 5 µm MIXED-C	7.5 x 300	PL1110-6500
PLgel 5 µm MIXED-D	7.5 x 300	PL1110-6504
PLgel 3 µm MIXED-E	7.5 x 300	PL1110-6300

PLgel MIXED Guards

Size (mm)	Particle Size (µm)	Part No.
7.5 x 50	20	PL1110-1220
7.5 x 50	10	PL1110-1120
7.5 x 50	5	PL1110-1520
7.5 x 50	3	PL1110-1320

Starches

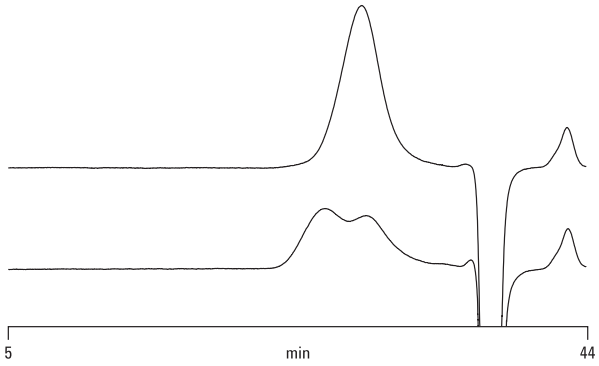
Column: **4 x PLgel 20 µm MIXED-A**
PL1110-6200
7.5 x 300 mm

Mobile Phase: DMSO + 5 mM $NaNO_3$

Flow Rate: 1.0 mL/min

Temperature: 80 °C

Detector: RI



Polyphenylene Sulfides

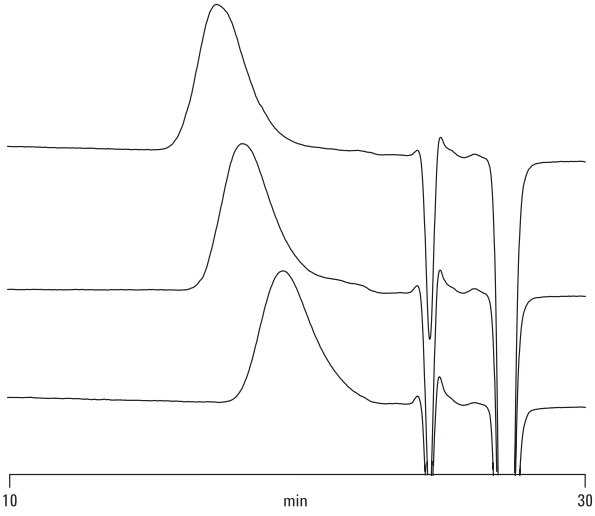
Column: **3 x PLgel 10 µm MIXED-B**
PL1110-6100
7.5 x 300 mm

Mobile Phase: o-Chloronaphthalene

Flow Rate: 1.0 mL/min

Temperature: 210 °C

Detector: RI



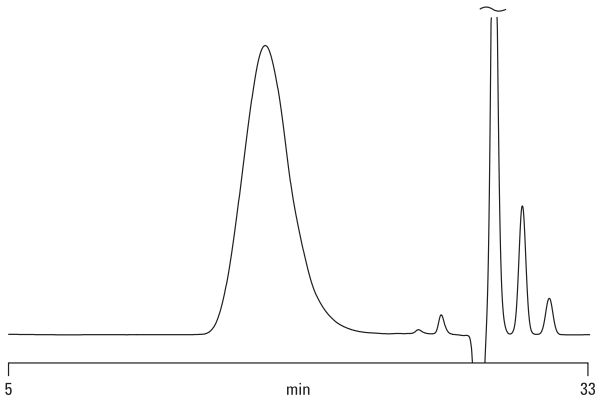
Plasticized PVC

Column: **3 x PLgel 5 µm MIXED-C**
PL1110-6500
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: RI



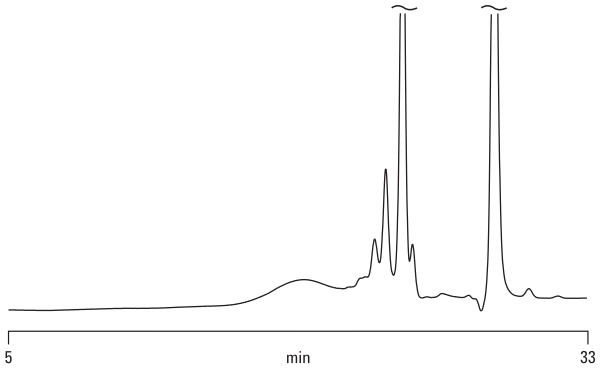
Epoxy Resin

Column: **3 x PLgel 5 µm MIXED-D**
PL1110-6504
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: RI



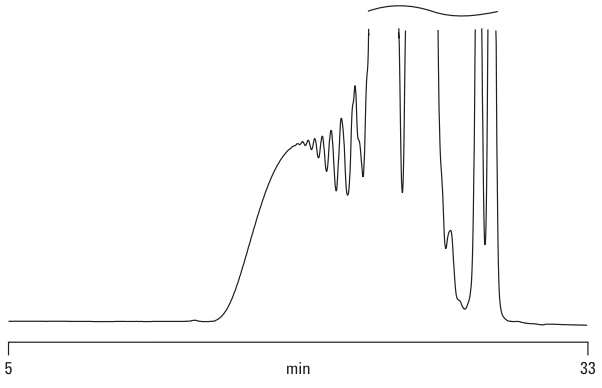
Polyol

Column: **PLgel 3 µm MIXED-E**
PL1110-6300
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm



PLgel MIXED-LS Columns

- Obtain an instant improvement in data quality
- No need for conditioning, saving time and solvent costs
- Maximize the potential of light scattering detectors

The PLgel MIXED-LS series is a PS/DVB packing using an innovative proprietary suspension polymerization technique to virtually eliminate nano-particle leakage. A startling improvement is achieved immediately in the quality of light scattering data obtained with PLgel MIXED-LS columns in place of conventional GPC columns. The light scattering chromatograms shown here were obtained after flushing the columns for one hour in THF at 1 mL/min. A polystyrene standard (Mp 210,000) was injected at 1 mg/mL in order to illustrate the dramatic improvement in signal-to-noise with the PLgel MIXED-LS column.

The performance of PLgel MIXED-LS columns has been matched to PLgel 20 µm MIXED-A and PLgel 10 µm MIXED-B columns in terms of calibration, column efficiency, wide solvent compatibility, and operating temperature. MIXED-LS are also ideal for online viscosity detection, minimizing the risk of capillary blockage, and can be used with regular PLgel guard columns that are packed with rigid low pore size gels with no particle bleed.

PLgel MIXED-LS Columns

Description	Size (mm)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
PLgel 10 µm MIXED-B LS	7.5 x 300	500-10,000,000	>35,000	PL1110-6100LS
PLgel 10 µm guard	7.5 x 50			PL1110-1120
PLgel 20 µm MIXED-A LS	7.5 x 300	2,000-40,000,000	>17,000	PL1110-6200LS
PLgel 20 µm guard	7.5 x 50			PL1110-1220

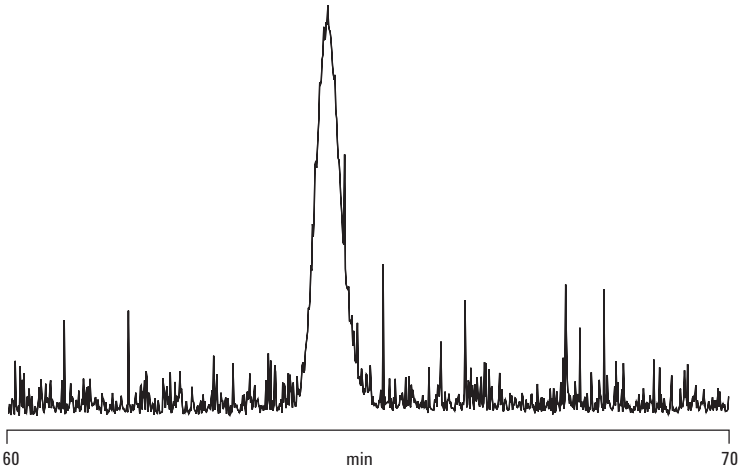
Conventional GPC column

Column: **Conventional GPC column**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: LS



Light scattering detection with a conventional GPC column – noise due to particulate bleed.

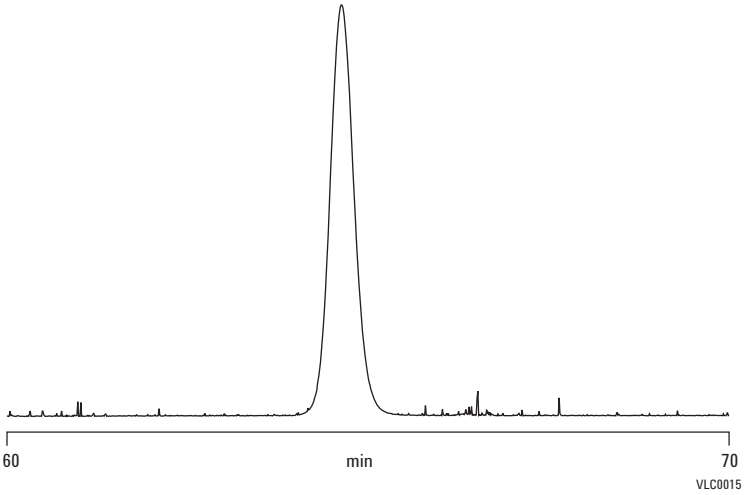
PLgel LS column

Column: **PLgel 10 µm MIXED-B LS**
PL1110-6100LS
7.5 x 300 mm, 10 µm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: LS



Light scattering detection with a PLgel LS column – minimal particulate bleed gives greatly improved baseline.

PLgel MiniMIX Columns

- Use about 70% less solvent and save money
- Store less solvent and increase operator safety
- High performance comparable to Agilent's conventional id columns

For reduced solvent cost and consumption, use industry standard PLgel MiniMIX mixed gel columns in 250 x 4.6 mm narrow bore dimensions. These narrow bore columns offer high performance, excellent solvent compatibility and mechanical stability. PLgel MiniMIX columns can be used with conventional GPC equipment.

To maintain the same linear velocity through the column, the volumetric flow rate must be reduced to 0.3 mL/min in line with the column cross sectional area, resulting in significantly lower solvent consumption. Sample loading should also be scaled down in line with reduced column volume, and system dead volume should be minimized to avoid excessive band broadening.

PLgel MiniMIX Columns

Description	Size (mm)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
PLgel 20 µm MiniMIX-A	4.6 x 250	2,000-40,000,000	> 17,000	PL1510-5200
PLgel 20 µm MiniMIX-A guard	4.6 x 50			PL1510-1200
PLgel 10 µm MiniMIX-B	4.6 x 250	500-10,000,000	> 35,000	PL1510-5100
Plgel 10 µm MiniMIX-B guard	4.6 x 50			PL1510-1100
PLgel 5 µm MiniMIX-C	4.6 x 250	200-2,000,000	> 50,000	PL1510-5500
PLgel 5 µm MiniMIX-C guard	4.6 x 50			PL1510-1500
PLgel 5 µm MiniMIX-D	4.6 x 250	200-400,000	> 50,000	PL1510-5504
PLgel 5 µm MiniMIX-D guard	4.6 x 50			PL1510-1504
PLgel 3 µm MiniMIX-E	4.6 x 250	up to 30,000	> 70,000	PL1510-5300
PLgel 3 µm MiniMIX-E guard	4.6 x 50			PL1510-1300

PLgel Individual Pore Size Columns

- Very high efficiency improves productivity
- Choose the optimum column for a perfect match of performance and application
- Fast analysis with fewer columns saves time and money

Individual pore size GPC columns offer high resolution over a specific molecular weight range. The linear portion of the calibration curve, where the slope is at its shallowest, defines the MW region over which optimum resolution will be achieved.

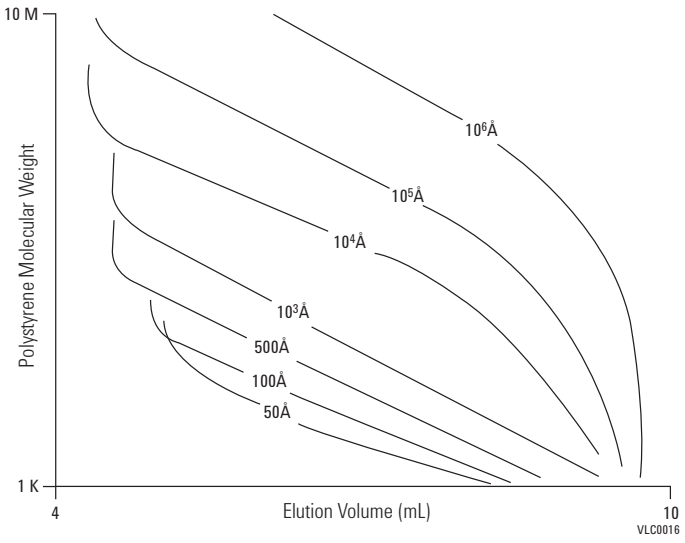
PLgel Individual Pore Size Columns

Size (mm)	Particle Size (µm)	Pore Size (Å)	Linear MW Operating Range (g/mol) (PS)	Guaranteed Efficiency (p/m)	Part No.
7.5 x 300	3	100	up to 4,000	> 100,000	PL1110-6320
7.5 x 300	5	50	up to 2,000	> 60,000	PL1110-6515
7.5 x 300	5	100	up to 4,000	> 60,000	PL1110-6520
7.5 x 300	5	500	500-30,000	> 60,000	PL1110-6525
7.5 x 300	5	10^3	500-60,000	> 50,000	PL1110-6530
7.5 x 300	5	10^4	10,000-600,000	> 50,000	PL1110-6540
7.5 x 300	5	10^5	60,000-2,000,000	> 50,000	PL1110-6550
7.5 x 300	10	50	up to 2,000	> 35,000	PL1110-6115
7.5 x 300	10	100	up to 4,000	> 35,000	PL1110-6120
7.5 x 300	10	500	500-30,000	> 35,000	PL1110-6125
7.5 x 300	10	10^3	500-60,000	> 35,000	PL1110-6130
7.5 x 300	10	10^4	10,000-600,000	> 35,000	PL1110-6140
7.5 x 300	10	10^5	60,000-2,000,000	> 35,000	PL1110-6150
7.5 x 300	10	10^6	600,000-10,000,000	> 35,000	PL1110-6160

PLgel Guard Column information can be found on page 500

Calibration curves

Calibrant: Polystyrene
Mobile Phase: THF
Flow Rate: 1.0 mL/min



PLgel Preparative Columns

- Excellent column efficiency provides optimum resolution
- High loading can isolate mg amounts for further study
- Over 10 times scale up permits efficient quantification

Preparative GPC is generally employed to fractionate polymers, isolate components in a polymer formulation or simplify mixtures of relatively small molecules in complex matrices. Mixtures of materials are easily separated on the basis of size, preferably in a low boiling organic solvent. They are then collected as a series of discrete fractions and isolated by simple evaporation of the solvent.

PLgel preparative columns are packed with the same rigid, high performance media as the analytical columns. The 10 µm particle provides high column efficiency (> 25,000 p/m) for optimum resolution and loading characteristics. PLgel 25 mm id preparative columns offer over 10 times scale-up compared to the 7.5 mm analytical columns. The increased id and column volume permit even higher loading. With low molecular weight materials, sample concentration can also be significantly increased, enabling production of milligram quantities of very pure material. The actual loading is ultimately controlled by the sample and its molecular weight.

PLgel Preparative Columns

Size (mm)	Particle Size (µm)	Pore Size (Å)	Linear MW Operating Range (g/mol) (PS)	Part No.
25 x 300	10	50	up to 2,000	PL1210-6115
25 x 300	10	100	up to 4,000	PL1210-6120
25 x 300	10	500	500-30,000	PL1210-6125
25 x 300	10	10^3	500-60,000	PL1210-6130
25 x 300	10	10^4	10,000-600,000	PL1210-6140
25 x 300	10	10^5	60,000-2,000,000	PL1210-6150
25 x 300	10	10^6	600,000-10,000,000	PL1210-6160
MIXED-B 25 x 300	10		500-10,000,000	PL1210-6100
MIXED-D 25 x 300	10		200-400,000	PL1210-6104
Prep guard 25 x 25				PL1210-1120

Columns for Special GPC/SEC Applications

EnviroPrep

- High sample loading ensures effective trace analysis
- Simple clean-up procedure saves sample preparation costs
- Optimized particle size distribution provides high resolution

EnviroPrep columns permit a simple, one stage clean-up as part of a methodology to determine pesticides in many organic matrices. The higher molecular weight fractions such as lipids, polymers, natural resins and dispersed high molecular weight components are easily eliminated in the GPC analysis.

Preparative GPC for soil extract clean-up is described in EPA Method 3640A using 300 x 25 mm and 150 x 25 mm columns to give higher sample loading and fraction yields, which is particularly useful for low levels of pollutants. Low pore size EnviroPrep columns are ideal for this method. The columns have 10 µm particles with 100Å pore sizes for high resolution, with an exclusion limit of 4000 g/mol. The preparative columns offer good resolution and high loading through optimization of the particle size distribution.

EnviroPrep

Size (mm)	Part No.
21.2 x 150	PL1E10-3120EPA
25 x 150	PL1210-3120EPA
21.2 x 300	PL1E10-6120EPA
25 x 300	PL1210-6120EPA

Columns for sample clean-up

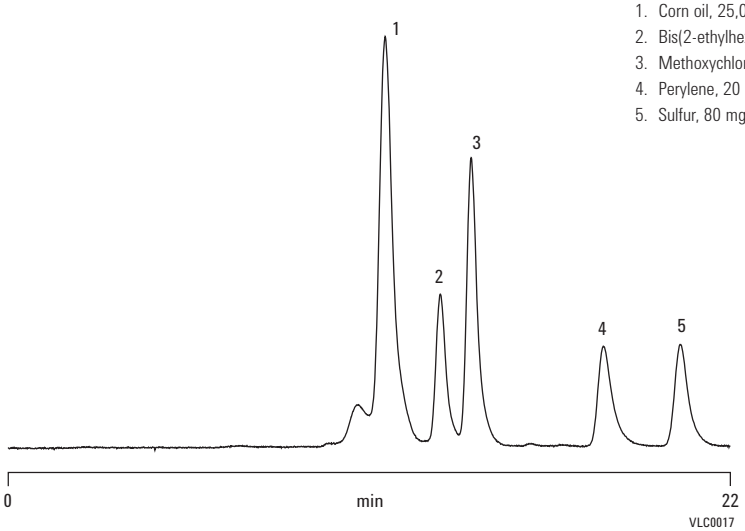
Column: **EnviroPrep PL1210-6120EPA 25 x 300 mm**

Column: **EnviroPrep PL1210-3120EPA 25 x 150 mm**

Mobile Phase: DCM

Flow Rate: 10 mL/min

Detector: UV, 254 nm



1. Corn oil, 25,000 mg/L
2. Bis(2-ethylhexyl) phthalate, 1,000 mg/L
3. Methoxychlor, 200 mg/L
4. Perylene, 20 mg/L
5. Sulfur, 80 mg/L

PLgel Olexis

- Optimized design for polyolefin analysis
- High temperature capability
- High resolution with no damage from sample shear provides clean separations

PLgel Olexis is designed for the analysis of very high molecular weight polymers, specifically polyolefins. The column resolves up to 100,000,000 g/mol (polystyrene in THF), and is packed with 13 µm particles to optimize efficiency and resolution without the risk of sample shear degradation during analysis. The packing of PLgel Olexis has the mechanical stability and robustness expected from a PLgel column, and so it is able to operate up to 220 °C for the analysis of highly crystalline materials.

PLgel Olexis

Description	Size (mm)	Part No.
PLgel Olexis	7.5 x 300	PL1110-6400
PLgel Olexis guard	7.5 x 50	PL1110-1400

PLgel Olexis reveals true modalities across the range of polyolefins

Column: **3 x PLgel Olexis PL1110-6400 7.5 x 300 mm**

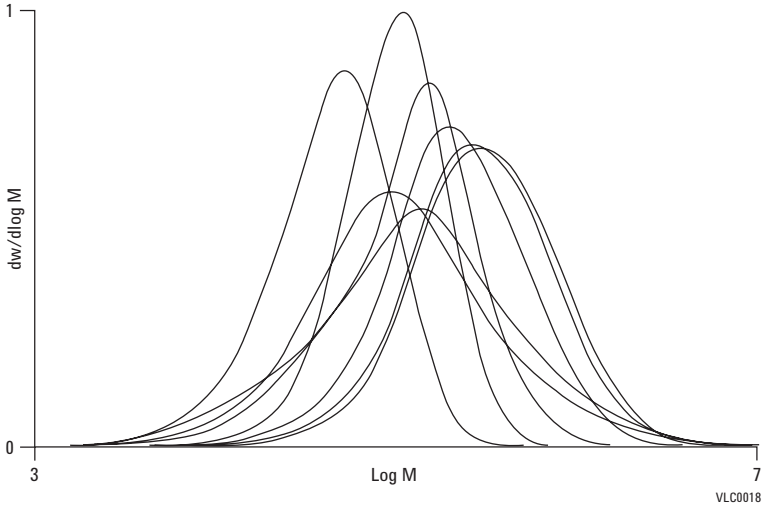
Mobile Phase: Trichlorobenzene + 0.0125% BHT

Flow Rate: 1.0 mL/min

Injection Volume: 200 µL

Temperature: 160 °C

Detector: PL-GPC 220 (RI)



PL HFIPgel

- Optimized separation range delivers high performance with no artifacts
- Highly durable packing prolongs column lifetime
- Low operating pressure reduces system wear and unnecessary downtimes

Hexafluoroisopropanol (HFIP) is used as a solvent in GPC for the analysis of important industrial polymers such as polyesters, polyamides and polylactide/glycolide copolymers. For greatly improved performance in extremely polar solvents such as HFIP and trifluoroethanol, we have developed novel "multipore" technology to produce PL HFIPgel, a PS/DVB packing featuring a monodisperse particle size, high pore volume, and high resolution.

Using PL HFIPgel avoids issues associated with conventional packings and HFIP, such as excessive curvature of calibration curves, dislocations/shoulders on peaks for polydisperse samples, and poor resolution in the low MW region.

Column efficiency is guaranteed > 30,000 p/m and the columns are very durable, with a maximum operating pressure of 145 bar (2030 psi). They are packed and tested in methanol but shipped ready-to-use in HFIP.

PL HFIPgel columns with 7.5 mm id normally operate at 1 mL/min. However, the 4.6 mm id columns run at 0.3 mL/min, providing a 70% reduction in solvent consumption with consequent savings in the cost of buying and disposing of solvents.

MW range for PL HFIPgel columns is 2,000,000 g/mol (PMMA in THF).

PL HFIPgel

Description	Size (mm)	Part No.
PL HFIPgel	4.6 x 250	PL1514-5900HFIP
PL HFIPgel	7.5 x 300	PL1114-6900HFIP
PL HFIPgel guard	7.5 x 50	PL1114-1900HFIP
PL HFIPgel guard	4.6 x 50	PL1514-1900HFIP

Polyamides

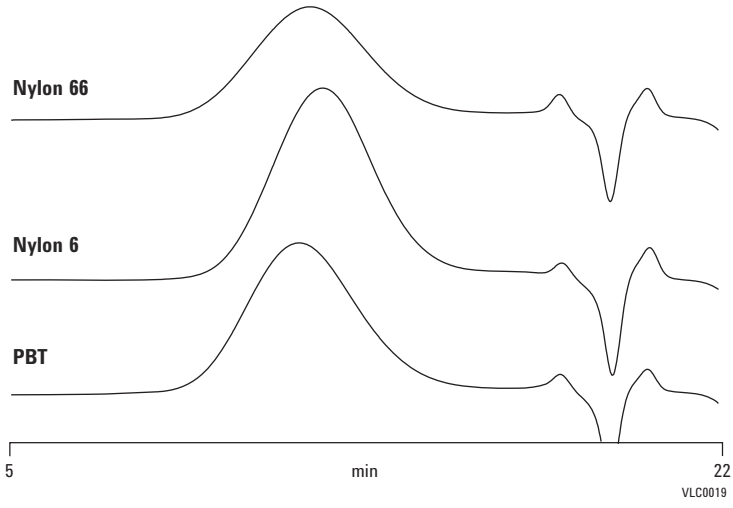
Column: **2 x PL HFIPgel PL1114-6900HFIP 7.5 x 300 mm**

Mobile Phase: HFIP + 20mM NaTFAc

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: PL-GPC 50 (RI)



PL Rapide

- Analysis in less than ten minutes saves time
- Significantly increased sample throughput improves efficiency
- Reduced solvent consumption and disposal costs save money
- Available in L, M, and H versions for low, medium, and high molecular weights; available in F version for flow injection analysis

Rapid GPC is an excellent tool for screening polymer MWD for trend analysis. Short PL Rapide columns reduce analysis times while maintaining the excellent solvent compatibility and mechanical stability of all GPC columns from Agilent.

PL Rapide columns are ideal for high speed applications such as high throughput screening, process monitoring, or tracking changes in MW distributions, where time is the most critical factor in the analysis. Packed with high quality gels, these columns cover the complete spectrum of molecular weights and are available for the analysis of both organic and water soluble polymers. Key features include high pore volume, high resolution packing materials, no special system requirements, choice of molecular weight resolving range, wide solvent compatibility, and excellent mechanical stability.

PL Rapide

Description	Size (mm)	MW Range (g/mol)	Guaranteed Efficiency (p/m)	Part No.
PL Rapide H	7.5 x 150	500-10,000,000	> 35,000	PL1113-3100
	10 x 100			PL1013-2100
PL Rapide M	7.5 x 150	200-2,000,000	> 60,000	PL1113-3500
	10 x 100			PL1013-2500
PL Rapide L	7.5 x 150	200-400,000	> 80,000	PL1113-3300
	10 x 100			PL1013-2300
PL Rapide F	7.5 x 150	up to 4,500	> 55,000	PL1113-3120
	10 x 100	up to 4,500	> 40,000	PL1013-2120
PL Rapide Aqua H	7.5 x 150	100-10,000,000	> 35,000	PL1149-3800
	10 x 100			PL1049-2800
PL Rapide Aqua L	7.5 x 150	100-30,000	> 35,000	PL1120-3830
	10 x 100			PL1020-2830

Resin analysis by rapid GPC

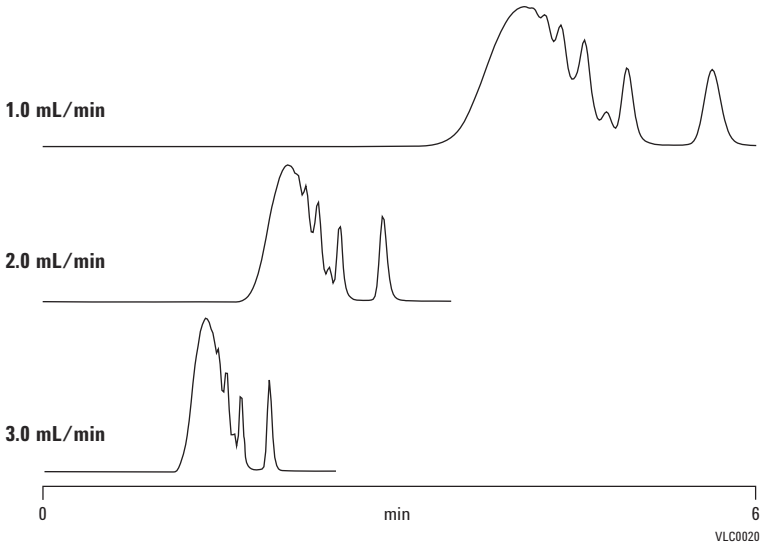
Column: **PL Rapide L**
PL1013-2300
10 x 100 mm

Sample: Epoxy resin

Mobile Phase: THF

Flow Rate: 1.0 , 2.0 and 3.0 mL/min

Detector: UV, 254 nm



PolarGel

- Medium polarity surface and high mechanical stability
- Operate in a wide range of solvents and solvent combinations
- Available in two resolving ranges, PolarGel-L and PolarGel-M

The PolarGel range is ideal for use with polar solvents, such as dimethyl formamide (DMF) and dimethyl sulfoxide (DMSO), and for solvent combinations such as tetrahydrofuran with water. These eluents are very useful in GPC/SEC to separate polar materials, such as polar resins, modified polysaccharides or complex polar polymers that are difficult to analyze in traditional SEC solvents, such as tetrahydrofuran alone. PolarGel-L is used for low molecular weight polar polymers and PolarGel-M for high MW polar polymers.

With polar polymers, highly polar groups can lead to non-specific interactions and secondary separation mechanisms when using polar solvents and traditional non-polar styrene/divinylbenzene columns. Additives and/or column conditioning are normally required to reduce these interactions. PolarGel has no need for these interventions, and also avoids the interactions and secondary effects that produce chromatogram distortions.

These PolarGel "mixed bed" columns have a medium polarity surface and high mechanical stability. They are capable of operating in a wide range of solvents and solvent combinations, greatly enhancing your ability to analyze polar polymers that are not necessarily water soluble. PolarGel is available in two resolving ranges to meet your precise requirements.

PolarGel

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Part No.
PolarGel-L	7.5 x 300	Up to 30,000	PL1117-6830
PolarGel-L guard	7.5 x 50		PL1117-1830
PolarGel-L repair gel			PL1417-0830
PolarGel-M	7.5 x 300	Up to 2,000,000	PL1117-6800
PolarGel-M guard	7.5 x 50		PL1117-1800
PolarGel-M repair gel			PL1417-0800

Two samples of melamine resin analyzed by PolarGel-L

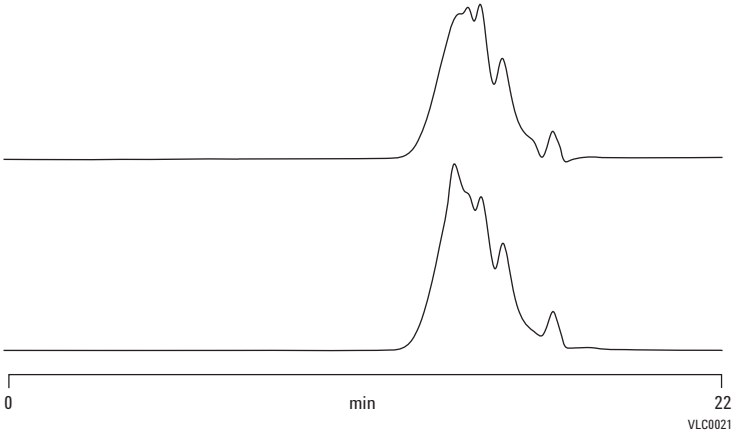
Column: **2 x PolarGel-L, 300 x 7.5 mm PL1117-6830**

Mobile Phase: Dimethyl acetamide + 0.1% LiBr

Flow Rate: 1.0 mL/min

Injection Volume: 100 µL

Detector: Agilent PL-GPC 220 (RI)



Excellent separation of two phenol formaldehyde resins with PolarGel-M

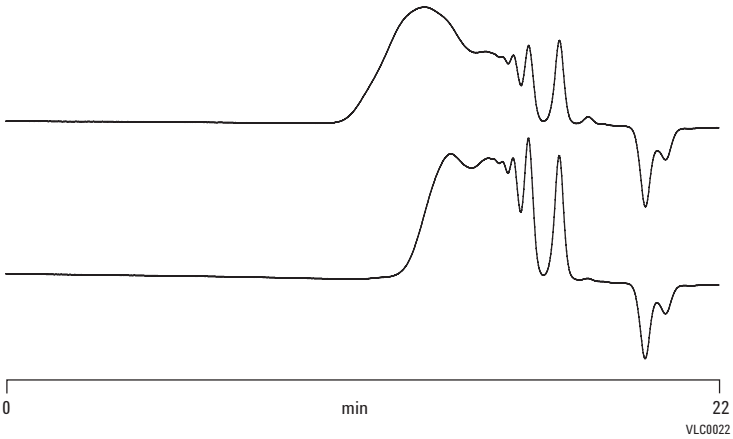
Column: **2 x PolarGel-M, 300 x 7.5 mm PL1117-6800**

Mobile Phase: 0.2% (w/v) DMF & 0.1% LiBr to reduce sample aggregation

Flow Rate: 1.0 mL/min

Injection Volume: 100 µL

Detector: Agilent PL-GPC 50 (RI)



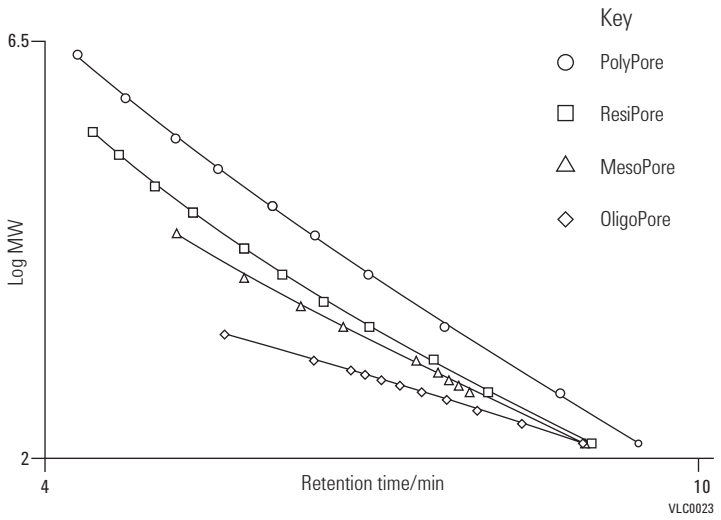
PlusPore

The PlusPore range has an increased pore volume that provides high resolution for specific applications. The high stability media permits the use of a wide range of organic solvents with accuracy and precision so that there is no distortion of the MW distribution shape.

The PlusPore series of columns has been specifically designed for high resolution GPC, and represents the very latest in GPC column technology. These novel packing materials are based on the industry standard, highly cross-linked polystyrene/divinylbenzene (PS/DVB), for the widest applicability and solvent compatibility. Each is made using a novel polymerization process to produce particles that exhibit a specific, controlled pore structure for optimum GPC performance. Typical applications include resins, condensation polymers, prepolymers, and oligomers.

For high resolution polymer analysis, the PolyPore, ResiPore, MesoPore, and OligoPore columns of the PlusPore product series exhibit a wide pore size distribution with near linear calibration curves covering an extended molecular weight range. These so-called "multipore" structures have increased pore volume compared to regular PS/DVB packing materials. This results in very high resolution GPC columns designed for specific application areas. The highly cross-linked porous particles provide excellent chemical and physical stability and permit easy transfer across the full range of organic solvents with little change in the shape of the calibration curve or the efficiency of the columns. As this multipore column technology does not require the combination of individual pore size packing materials, the result is high accuracy and precision without any artifacts in the shape of the molecular weight distribution.

PlusPore calibration curves



PlusPore Selection Guide

Column	MW Range (g/mol) (PS)	Nominal Particle Size (µm)	Typical Efficiency (p/m)	Recommended Calibrants	Frit Porosity (µm)
PolyPore	200-2,000,000	5	> 60,000	EasiCal PS-1or EasiVial PS-H	2
ResiPore	200-400,000	3	> 80,000	EasiCal PS-2 or EasiVial PS-M	2
MesoPore	up to 25,000	3	> 80,000	Polystyrene S-L-10 Kit	2
OligoPore	up to 4,500	6	> 55,000	Polystyrene S-L2-10 Kit	2

PolyPore

- Routine polymer analysis with very high resolution
- Wide operating range simplifies column choice
- Low particle size extracts maximum information from the analyte

PolyPore columns have been specifically developed to give unrivaled resolution for the analysis of polymers with broad molecular weight distributions. With a wide operating range covering many decades of molecular weight, PolyPore columns combine a 5 µm particle size with extremely high pore volume to give the highest possible resolution, ensuring the most detailed information possible from your analysis.

PolyPore

Description	Size (mm)	Part No.
PolyPore	7.5 x 300	PL1113-6500
PolyPore guard	7.5 x 50	PL1113-1500

Comparison of PolyPore with conventional individual pore size GPC columns

Column A: **2 x PolyPore PL1113-6500 7.5 x 300 mm**

Column B: **PLgel 10^3Å 7.5 x 300 mm, 5 µm**

Column C: **PLgel 10^5Å 7.5 x 300 mm, 5 µm**

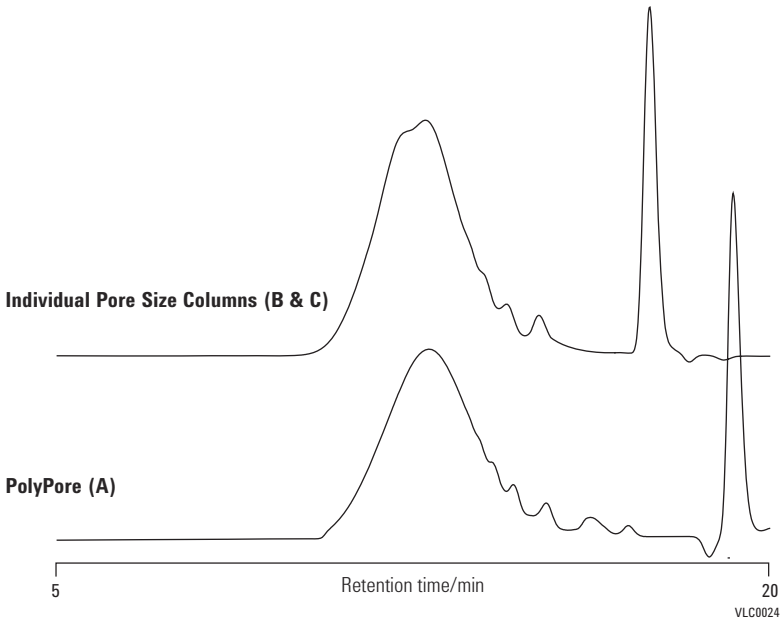
Sample: High MW Resin

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume 100 µL

Detector: Agilent PL-GPC 50 (RI)



Polymethylmethacrylate in DMF

Column: **2 x PolyPore PL1113-6500 7.5 x 300 mm**

Sample: Commercial PMMA

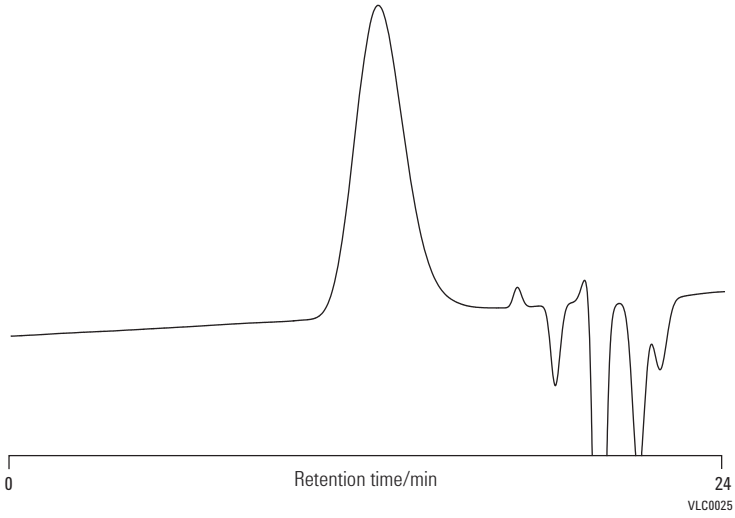
Mobile Phase: DMF + 0.1% LiBr

Flow Rate: 1.0 mL/min

Temperature: 80 °C

Injection Volume: 100 µL

Detector: Agilent PL-GPC 50 (RI)



ResiPore

- Efficient separation of complex molecular weight distributions
- Reveals oligomer content to provide a true representation of the sample
- High pore volume extracts maximum information from the analyte

ResiPore columns are the ideal choice for the analysis of resins and condensation polymers with complex molecular weight distributions that include oligomer content. By combining a 3 µm particle size and high pore volume, high efficiency ResiPore columns offer maximum resolution of these intermediate molecular weight polymers.

ResiPore

Description	Size (mm)	Part No.
ResiPore	7.5 x 300	PL1113-6300
ResiPore guard	7.5 x 50	PL1113-1300

Alkyd resin

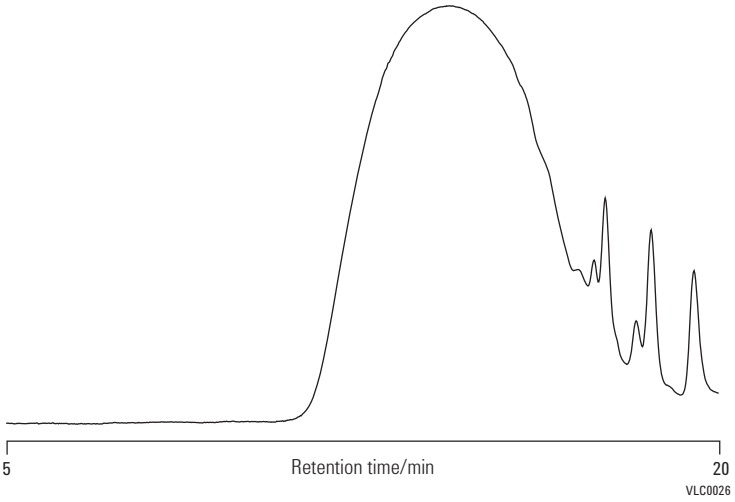
Column: **2 x ResiPore PL1113-6300 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: UV, 254 nm



Polyester

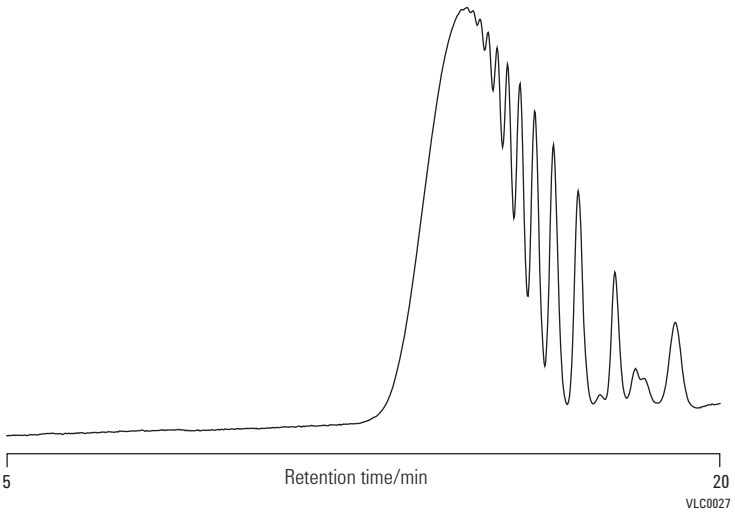
Column: **2 x ResiPore PL1113-6300 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: UV, 254 nm



MesoPore

- Full solvent compatibility with no detrimental effect on efficiency
- Low particle size extracts maximum information from the analyte
- No MWD dislocations so the distribution is an accurate representation of the sample

MesoPore columns have been specifically designed to provide optimum results in the analysis of prepolymers, i.e. polymeric materials with a large oligomeric component. By combining a 3 µm particle size with high pore volume, MesoPore columns give the highest resolution separations for the analysis of low molecular weight polymers, such as prepolymers, resins, polyols, and siloxanes.

MesoPore

Description	Size (mm)	Part No.
MesoPore	7.5 x 300	PL1113-6325
MesoPore guard	7.5 x 50	PL1113-1325

Polyurethanes

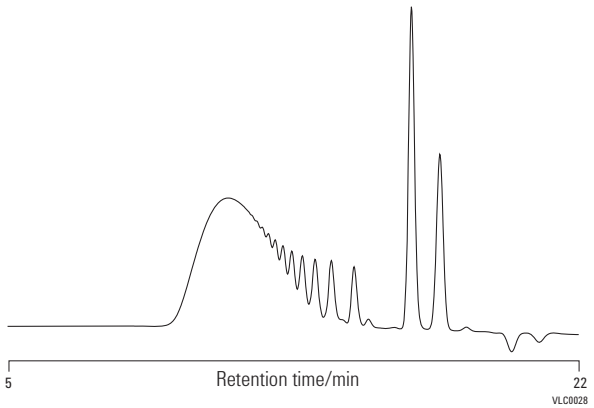
Column: **2 x MesoPore PL1113-6325 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: Agilent PL-GPC 50 (RI)



Polyesterimide

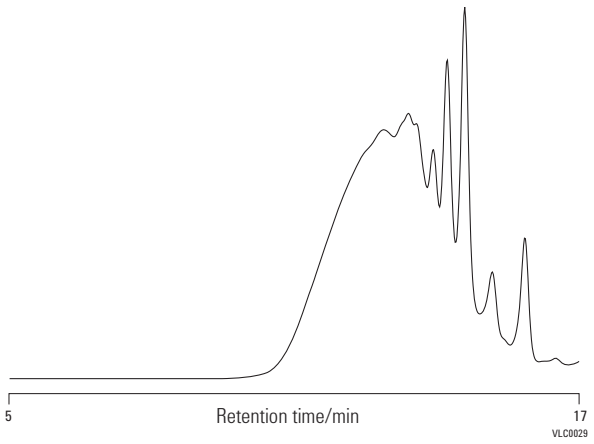
Column: **2 x MesoPore PL1113-6325 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: Agilent PL-GPC 50 (RI)



OligoPore

- Near linear calibration curve for best accuracy and precision
- Very stable media allows for a wide choice of solvents
- Isolation of individual fractions reveals more information from whole samples

OligoPore columns have been developed from an innovative new media that exhibits significantly increased pore volumes compared to conventional low pore size GPC columns. The outcome is higher resolution in the oligomeric region. The 300 x 25 mm preparative column offers high resolution at greatly increased loading for effective isolation of individual components. Oligomer fractions collected from the OligoPore preparative column can then be re-injected on analytical columns to check for the purity of the fractions and for comparison with the whole sample.

OligoPore

Description	Size (mm)	Part No.
OligoPore	25 x 300	PL1213-6520
OligoPore	7.5 x 300	PL1113-6520
OligoPore guard	7.5 x 50	PL1113-1320

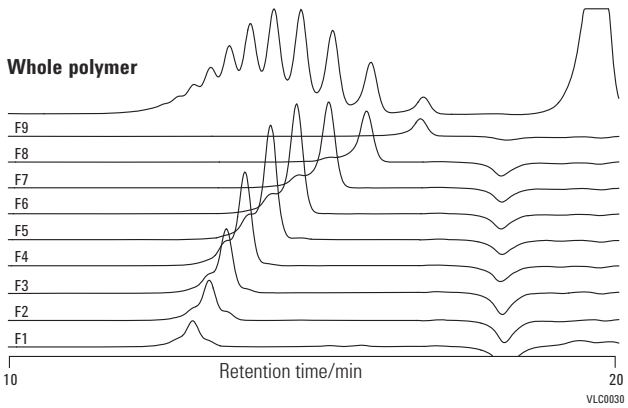
Analysis of low molecular weight polystyrene and oligomer fractions collected from OligoPore preparative columns

Column: **2 x OligoPore PL1113-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: UV



Analytical separation of low molecular weight polystyrene

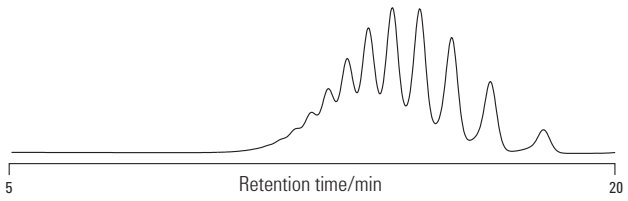
Column: **2 x OligoPore PL1213-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Loading: 0.2%, 100 mL

Detector: UV



Preparative separation of low molecular weight polystyrene

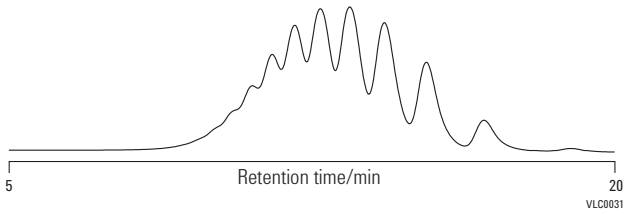
Column: **2 x OligoPore PL1213-6520 25 x 300 mm**

Mobile Phase: THF

Flow Rate: 10.0 mL/min

Loading: 2.0%, 2 mL

Detector: UV



Aqueous SEC of Polymers

PL aquagel-OH SEC

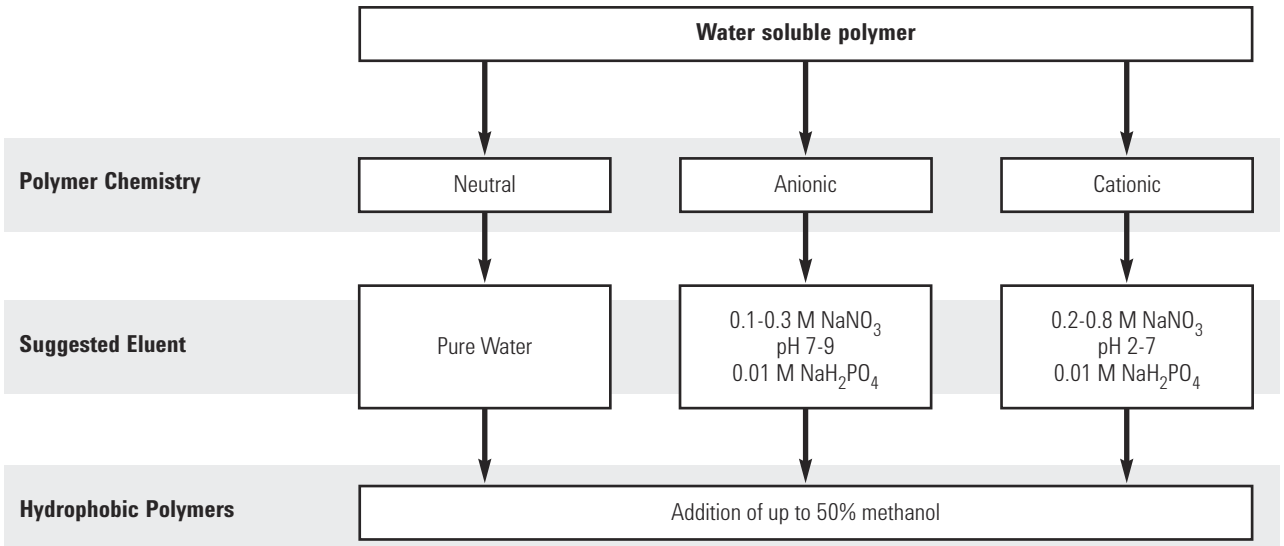
Aqueous size exclusion chromatography (SEC) is widely used for the determination of molecular weight distributions of a variety of synthetic and naturally occurring water-soluble polymers, and separations of oligomers and small molecules. The requirement to eliminate ionic and hydrophobic effects makes aqueous SEC very demanding.

The PL aquagel-OH series provides a chemically and physically stable matrix for reliable aqueous SEC separations. The columns are packed with macroporous copolymer beads with an extremely hydrophilic polyhydroxyl functionality. The "neutral" surface and the capability to operate across a wide range of eluent conditions provide for high performance analyses of compounds with neutral, ionic, and hydrophobic moieties, alone or in combination. PL aquagel-OH is available for analytical and preparative applications.

Optimizing Conditions for Aqueous SEC with PL aquagel-OH Columns

Due to the complex nature of water-soluble polymers, it is often necessary to modify the eluent in order to avoid sample-to-sample and sample-to-column interactions which can result in poor aqueous SEC separations. The excellent stability of the PL aquagel-OH packing material allows the eluent to be tailored to suit the polymer, while retaining the high column efficiency. For ionic interactions, the eluent can be modified by the addition of salt and/or the adjustment of pH. For water soluble polymers with a hydrophobic character, only the addition of a weak organic solvent (methanol) is required to inhibit hydrophobic interactions.

Guide to eluent selection for PL aquagel-OH applications



PL aquagel-OH Column Selection Guide

Sample Type	Typical Applications	Recommended Column Sets
Low MW polymers and oligomers	Surfactants, oligosaccharides, PEGs, lignosulfonates, polyacrylates	2 or 3, 30, 20 PL aquagel-OH 8 µm, or PL aquagel-OH 20 5 µm, or PL aquagel-OH MIXED-M 8 µm
Polydisperse synthetic or naturally occurring polymers	Polysaccharides, PVA, cellulose derivatives, PEO, polyacrylic acid	2 or 3 PL aquagel-OH MIXED-H 8 µm, or PL aquagel-OH 60/50/40 8 µm
Very high MW polymers	Polyacrylamides, hyaluronic acids, CMC, starches, gums	PL aquagel-OH 60/50/40 15 µm in series

PL aquagel-OH Analytical

- Highly stable matrix ensures reliable separations, even with modified eluents
- MIXED columns cover a wide range of molecular weights, simplifying column selection
- Highly versatile for neutral, polar, anionic and cationic samples

The PL aquagel-OH analytical series has a pH range of 2-10, compatibility with organic solvent (up to 50% methanol), mechanical stability up to 140 bar (2030 psi) and low column operating pressures.

PL aquagel-OH Analytical

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Guaranteed Efficiency (p/m)	Part No.
PL aquagel-OH 20 5 µm	7.5 x 300	100-20,000	> 5,000	PL1120-6520
PL aquagel-OH 20 8 µm	7.5 x 300	100-20,000	> 35,000	PL1149-6820
PL aquagel-OH 30 8 µm	7.5 x 300	100-30,000	> 35,000	PL1120-6830
PL aquagel-OH 40 8 µm	7.5 x 300	10,000-200,000	> 35,000	PL1149-6840
PL aquagel-OH 40 15 µm	7.5 x 300	10,000-200,000	> 15,000	PL1149-6240
PL aquagel-OH 50 8 µm	7.5 x 300	50,000-1,000,000	> 35,000	PL1149-6850
PL aquagel-OH 50 15 µm	7.5 x 300	50,000-1,000,000	> 15,000	PL1149-6250
PL aquagel-OH 60 8 µm	7.5 x 300	200,000-> 10,000,000	> 35,000	PL1149-6860
PL aquagel-OH 60 15 µm	7.5 x 300	200,000-> 10,000,000	> 15,000	PL1149-6260
PL aquagel-OH MIXED-H 8 µm	7.5 x 300	100-10,000,000	> 35,000	PL1149-6800
PL aquagel-OH MIXED-M 8 µm	7.5 x 300	Up to 600,000	> 35,000	PL1149-6801
PL aquagel-OH 10 µm guard	25 x 25			PL1249-1120
PL aquagel-OH 5 µm guard	7.5 x 50			PL1149-1530
PL aquagel-OH 8 µm guard	7.5 x 50			PL1149-1840

TIPS & TOOLS

Buffers in a stored column may crystallize and cause damage. Flush the column with water containing a small amount of sodium azide to prevent biological growth.

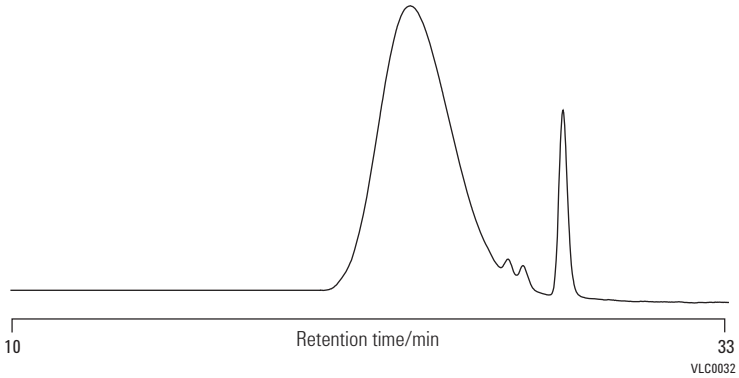
Polyvinyl alcohol

Column: **3 x PL aquagel-OH MIXED-H 8µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



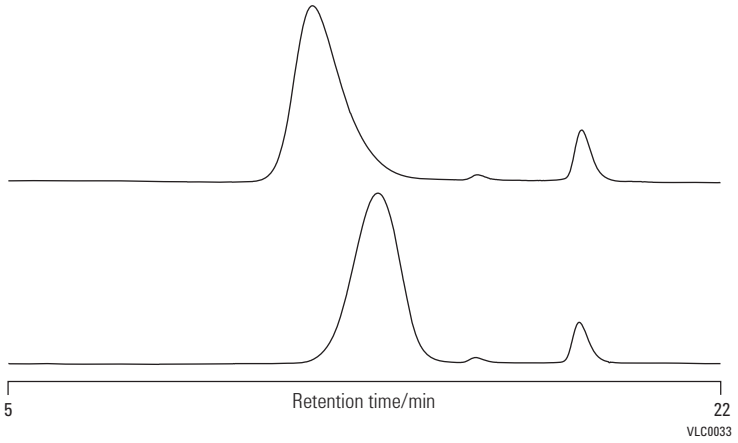
Heparin

Column: **2 x PL aquagel-OH 30 8 µm**
PL1120-6830
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Hyaluronic acid

Column: **PL aquagel-OH 60 15 µm**
PL1149-6260
7.5 x 300 mm

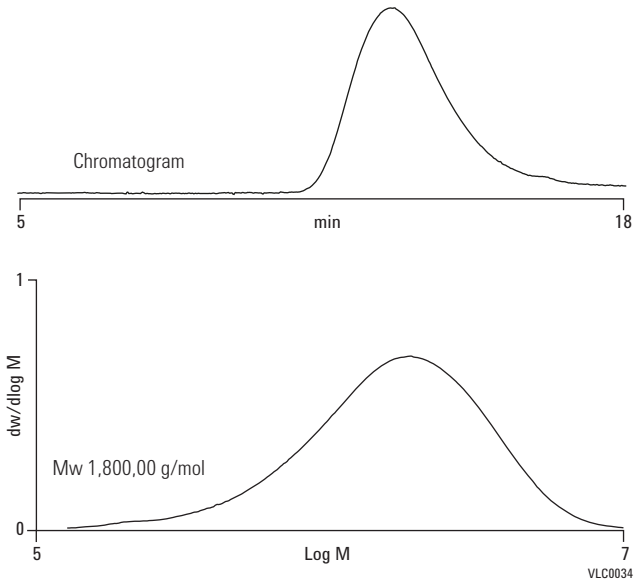
And

PL aquagel-OH 40 15 µm
PL1149-6240
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Differences in composition of two alkyl naphthalene sulfonates

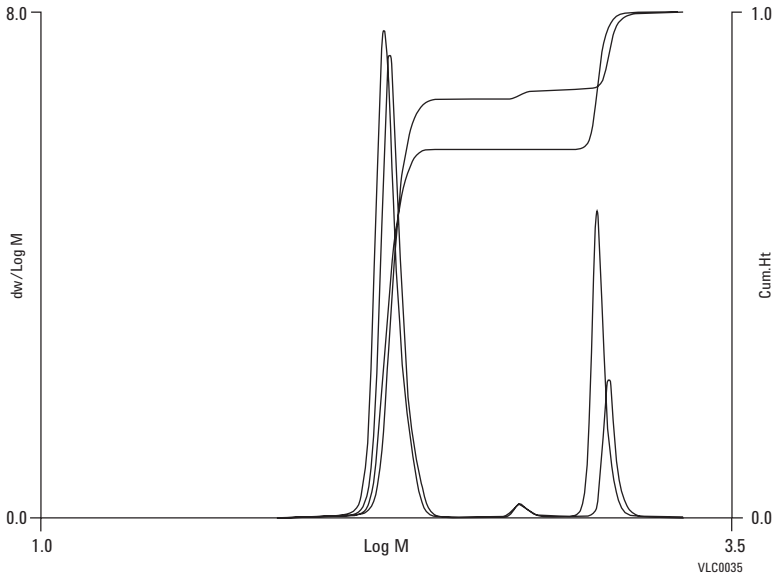
Column: **2 x PL aquagel-OH 20 5µm**
PL1120-6520
7.5 x 300 mm

Mobile Phase: 0.25 M ammonium formate in water

Flow Rate: 1.0 mL/min

Injection Volume: 20 µL

Detector: ELS (neb=30 °C, evap=30 °C, gas=1.4 SLM)



PL aquagel-OH Preparative

- Up to 10 times scale-up maximizes yield
- High loading maximizes sample throughput
- Carefully chosen particle size provides optimum resolution

Preparative SEC is used for the fractionation of a wide variety of water-soluble samples based on their size in solution. The technique is applied to the fractionation of disperse polymers or to isolate components in a polymer formulation.

Preparative PL aquagel-OH columns and associated guard columns enable rapid and convenient scale-up from analytical separations. The 25 mm id prep column offers at least a 10 times scale-up in loading from the 7.5 mm id analytical columns. Typically, a 10 mL/min flow rate results in a separation time of ten minutes with a 300 mm column. The columns are packed with the same robust macroporous particles as the analytical column range. The 8 µm particle size provides optimum resolution and loading characteristics with column efficiency > 20,000 plates/m.

PL aquagel-OH Preparative

Description	Size (mm)	MW Range (g/mol) (PEG/PEO)	Part No.
PL aquagel-OH 30 8 µm	25 x 300	100-30,000	PL1220-6130
PL aquagel-OH 40 8 µm	25 x 300	10,000-200,000	PL1249-6140
PL aquagel-OH 50 8 µm	25 x 300	50,000-1,000,000	PL1249-6150
PL aquagel-OH MIXED 8 µm	25 x 300	100-10,000,000	PL1249-6100
PL aquagel-OH 10 µm guard	25 x 25		PL1249-1120

Polyvinyl alcohol

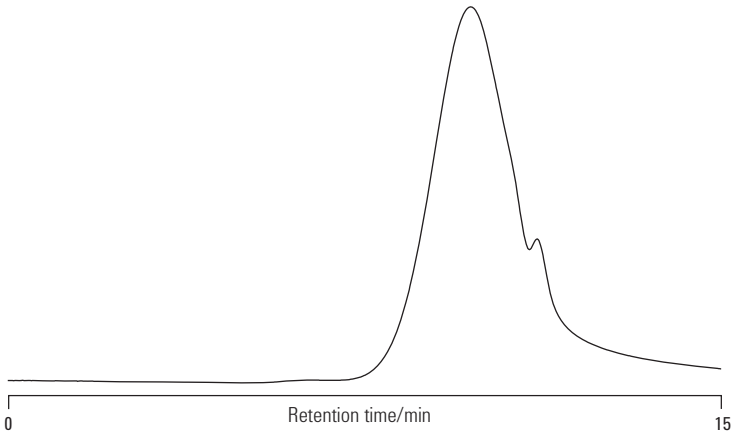
Column: **PL aquagel-OH 40 8 µm PL1249-6140 25 x 300 mm**

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 10.0 mL/min

Loading: 10 mg/mL, 2 mL

Detector: Agilent PL-GPC 50 (RI)



GPC Column Accessories

Description	Unit	Part No.
Frit removal tool for threaded columns only	1/pk	PL1310-0001
2 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0002
5 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0012
10 µm frit kit for threaded columns, 7.5 mm id	5/pk	PL1310-0036
PLgel column repair gel, 10 µm	1/pk	PL1410-0101
PLgel column repair gel, 5 µm	1/pk	PL1410-0501
Column connecting nuts, 1/16 in tube	5/pk	PL1310-0007
Tubing ferrules, 1/16 in tube	5/pk	PL1310-0008
Connecting tubing, 10 cm length, 0.01 in id	10/pk	PL1310-0048
LDV intercolumn stainless steel connector	1/pk	PL1310-0005
PLgel column repair gel, 3 µm	1/pk	PL1410-0301
PLgel Olexis column repair gel	1/pk	PL1410-0200

Polymer Standards for GPC/SEC

Polymer standards from Agilent are the ideal reference materials for generating accurate, reliable GPC/SEC column calibrations, with the assurance of the ISO 9001:2000 quality standard. Additional applications for our highly characterized homopolymers exhibiting unique characteristics are used as model polymers for research and analytical method development.

Agilent manufactures the highest quality polymer standards with extremely narrow polydispersity and the widest molecular weight range commercially available. These quality polymer standards are supplied with extensive characterization data utilizing a variety of independent techniques (e.g. light scattering and viscometry) and high performance GPC to verify polydispersity and assign that all important peak molecular weight (Mp).

Our comprehensive range of EasiVial, EasiCal, and traditional calibration kits has been specifically designed to cover all molecular weight ranges for organic and aqueous GPC/SEC applications. We provide you with the widest choice to maximize your specific characterization needs. In addition, we supply other polymers as individual molecular weights, and broad distribution polymers for system validation or broad standard calibration procedures.

Calibration Kits

Agilent offers a wide range of polymer standards kits for conventional GPC/SEC column calibration or for calibrating light scattering and viscometry detectors. The kits are in boxed sets of ten different polymer standards covering a particular molecular weight range, to be used with organic and aqueous, medium polarity, and polar solvents. Every individual polymer has its own Certificate of Analysis of the analytical conditions and values, such as Mp needed for constructing a calibration plot. The polymers are chosen to give equidistant calibration points on a logarithmic MW scale, providing a more uniform calibration curve.

Individual Polymer Molecular Weights

We design our individual standards to have the narrowest molecular weight distribution commercially available. Additionally, they cover the widest molecular weight range, from 162-15 million MW. The current polystyrene nominal molecular weight of 15 million MW has a polydispersity ≤1.10. These standards are generally available in 1, 5 and 10 g quantities, and each comes with its own Certificate of Analysis detailing analysis conditions and relevant data.

GPC/SEC Standards Selection Guide

Polymer Type	Individual MW	Calibration Kits	EasiCal	EasiVial	Type of GPC/SEC
Polystyrene	✓	✓	✓	✓	Organic
Polymethylmethacrylate	✓	✓		✓	Organic
Polyethylene glycol (PEG)	✓	✓		✓	Organic/Aqueous
Polyethylene oxide (PEO)	✓	✓		✓	Organic/Aqueous
Pullulan polysaccharide	✓	✓			Organic/Aqueous
Polyacrylic acid Na salt	✓	✓			Aqueous

EasiVial

- Eliminates tedious weighing procedures to improve calibration accuracy
- Reduces solvent dispensing to limit risks associated with handling solvents
- For conventional and multi detector GPC to maximize applicability

For organic and aqueous GPC/SEC column calibration, this premier product is the quickest and most convenient method to deliver an accurate 12-point column calibration.

The key to achieving baseline separation from polymer mixtures, and therefore eliminating doubt and errors, is in selecting only the narrowest polydispersity polymers. This is where Agilent polymer standards excel and deliver, as shown in the chromatograms.

The EasiVial standards kit is a pre-prepared, time saving product for rapid and reliable GPC column calibration. EasiVial kits contain three vials, each with a mixture of four accurately pre-weighed polymer standards, providing a 12-point GPC calibration in just three injections. The mass of each polymer in the vial is accurately known, so that upon addition of a fixed volume of eluent, the solution is prepared at a precise concentration. EasiVial is ideal for both conventional and multi detector GPC calibration. Simply prepare and manually inject, or transfer to autosampler vials, or place directly into a compatible autosampler.

Every EasiVial kit contains 30 vials (ten of each type) that are color-coded for easy identification and are available in 4 or 2 mL vials making them suitable for most autosamplers. The kits are available for polystyrene (PS), polymethylmethacrylate (PMMA), polyethylene glycol/oxide (PEG/PEO) and polyethylene glycol (PEG). For added value, a Tri-Pack (90 vials) is offered, extending reproducibility.

EasiVial PS-H

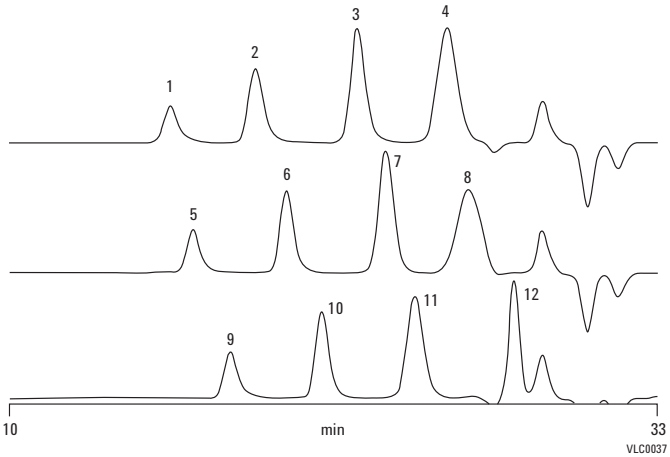
Column: **3 x PLgel 10 µm MIXED-B**
PL1110-6100
7.5 x 300 mm

Mobile Phase: THF

Flow Rate: 1.0 mL/min

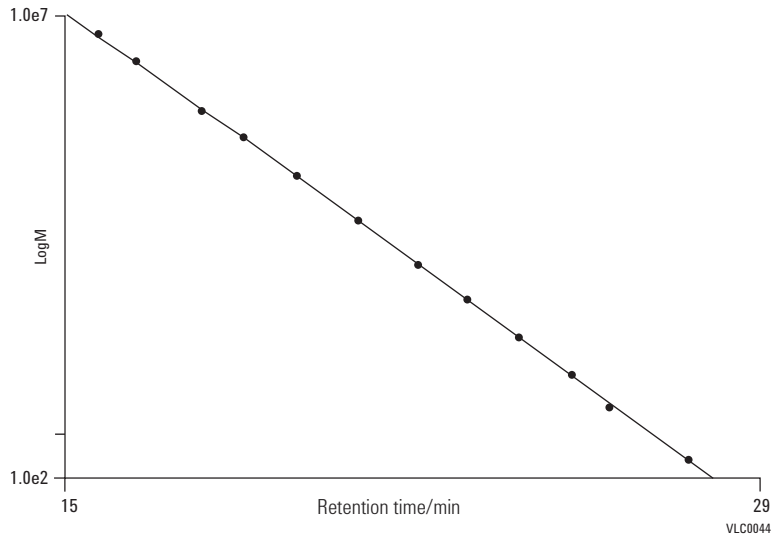
Temperature: 40 °C

Detector: PL-GPC 220 (RI)



1. 6,035,000
2. 483,000
3. 19,720
4. 1,260
5. 3,053,000
6. 84,900
7. 8,450
8. 580
9. 915,000
10. 60,450
11. 3,370
12. 162

Polystyrene calibration generated with EasiVials



Specifications

EasiVial Color	EasiVial PS-H	EasiVial PS-M	EasiVial PS-L	EasiVial PM	EasiVial PEG/PEO	EasiVial PEG
			Nominal Mp (g/mol)			
Red	1,300	780	580	2,000	600	282
	20,000	6,000	3,000	30,000	12,000	1,000
	500,000	50,000	10,000	300,000	125,000	6,000
	6,000,000	400,000	40,000	2,000,000	1,200,000	35,000
Yellow	580	370	370	1,000	200	194
	8,500	2,500	2,000	13,000	4,000	600
	185,000	25,000	6,000	150,000	60,000	3,750
	3,000,000	200,0001	25,000	800,000	1,000,000	21,000
Green	162	162	162	600	100	106
	3,400	1,500	1,000	5,700	1,500	420
	60,000	11,000	4,000	80,000	25,000	2,000
	900,000	100,000	16,000	470,000	460,000	12,000

Description Key

PS: Polystyrene
PM: Polymethylmethacrylate
PEG/PEO: Polyethylene Glycol/Oxide
H: High
M: Medium
L: Low

EasiVial Pre-weighed Calibration Kits

Description	Range of Nominal Mp (g/mol)	Vial Volume (mL)	Unit	Part No.
EasiVial PEG/PEO	100-1,200,000	2	30/pk	PL2080-0201
EasiVial PEG/PEO	100-1,200,000	4	30/pk	PL2080-0200
EasiVial PEG	106-35,000	2	30/pk	PL2070-0201
EasiVial PEG	106-35,000	4	30/pk	PL2070-0200
EasiVial PM	600-2,000,000	2	30/pk	PL2020-0201
EasiVial PM	600-2,000,000	4	30/pk	PL2020-0200
EasiVial PS-H	162-6,000,000	2	30/pk	PL2010-0201
EasiVial PS-H	162-6,000,000	4	30/pk	PL2010-0200
EasiVial PS-M	162-400,000	2	30/pk	PL2010-0301
EasiVial PS-M	162-400,000	4	30/pk	PL2010-0300
EasiVial PS-L	162-40,000	2	30/pk	PL2010-0401
EasiVial PS-L	162-40,000	4	30/pk	PL2010-0400
PEG/PEO Tri-Pack		2	90/pk	PL2080-0202
PEG/PEO Tri-Pack		4	90/pk	PL2080-0203
PEG Tri-Pack		2	90/pk	PL2070-0202
PEG Tri-Pack		4	90/pk	PL2070-0203
PMMA Tri-Pack		2	90/pk	PL2020-0202
PMMA Tri-Pack		4	90/pk	PL2020-0203
PS-H Tri-Pack		2	90/pk	PL2010-0202
PS-H Tri-Pack		4	90/pk	PL2010-0203
PS-L Tri-Pack		2	90/pk	PL2010-0402
PS-L Tri-Pack		4	90/pk	PL2010-0403

EasiCal

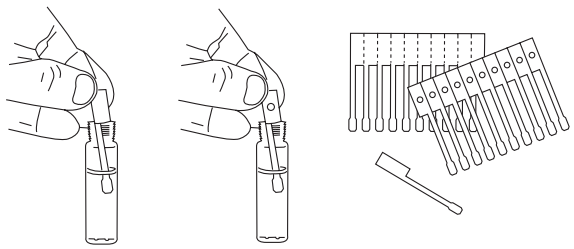
- Easy three-step process with no fuss
- Cost-effective format saves money
- Only two injections for improved productivity

The EasiCal system for organic solvents consists of two different combs, each with ten detachable spatulas, supporting a mixture of five polymer standards. The thin film of polymer (approximately 5 mg) on the tip of the PTFE spatulas rapidly dissolves when immersed in eluent to provide two GPC/SEC calibration solutions. A single pack provides ten spatulas of each type, with MWs selected to provide equidistant calibration points for greater accuracy.

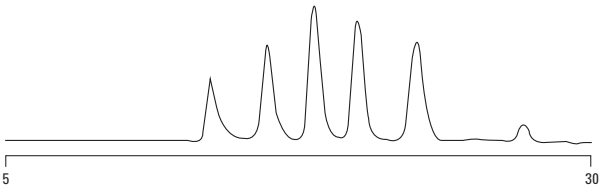
EasiCal Pre-prepared Polystyrene Kits

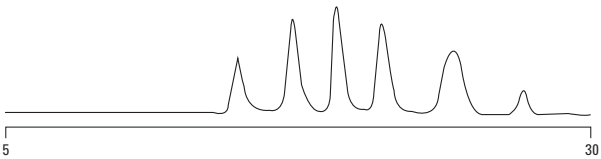
Description	Range of Nominal Mp (g/mol)	Unit	Part No.
Polystyrene PS-1	580-7,500,000	1/pk	PL2010-0501
		5/pk	PL2010-0505
Polystyrene PS-2	580-400,000	1/pk	PL2010-0601
		5/pk	PL2010-0605

Column calibration for GPC/SEC is as easy as 1, 2, 3...

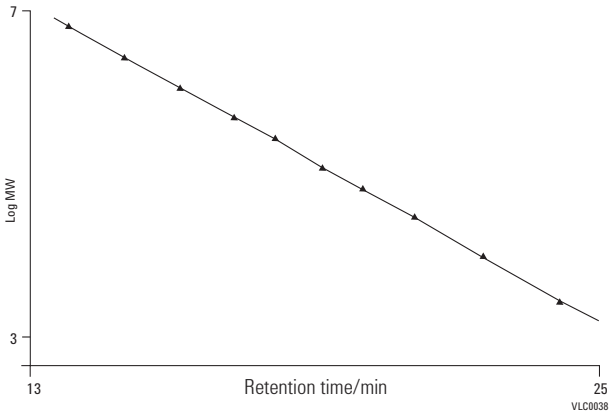


1. Place one spatula of each type into appropriate volume of solvent.





2. Chromatograph each solution; only two injections required



3. Generate a 10-point calibration

Polystyrene

- Compatible with most organic solvents
- Certificate of Analysis meets international protocols
- Calibration capability for virtually all applications

Polystyrene standards are the first choice for many organic solvents, either for conventional GPC column calibration or for calibrating light scattering and viscosity detectors. Our organic polymers cover a range from 162-15 million MW, with MWs selected to provide equidistant calibration points for greater accuracy. Every kit contains 0.5 g of ten different molecular weight standards.

Calibration Kits, (All Kits 10 x 0.5 g)

S-H-10 Part No. PL2010-0103	S-H2-10 Part No. PL2010-0104	S-M-10 Part No. PL2010-0100	S-M2-10 Part No. PL2010-0102	S-L-10 Part No. PL2010-0101	S-L2-10 Part No. PL2010-0105
Constituent Polymer Nominal Mp (g/mol)					
300,000	1,000	580	580	162	162
460,000	3,000	1,450	1,400	580	370
700,000	8,600	4,000	2,400	900	580
1,100,000	25,000	10,000	4,750	1,400	800
1,700,000	73,000	27,000	9,500	2,200	1,000
2,600,000	210,000	66,000	19,000	3,400	1,500
4,000,000	600,000	180,000	38,000	5,100	1,900
6,200,000	1,780,000	460,000	75,000	8,100	2,500
9,500,000	5,000,000	1,190,000	150,000	12,800	3,200
15,000,000	15,000,000	3,000,000	300,000	20,000	4,500

Description Key

H: High
M: Medium
L: Low

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
162	1.00	PL2012-1001	PL2012-1005	PL2012-1010
370	1.11	PL2012-0001	PL2012-0005	PL2012-0010
580	1.11	PL2012-2001	PL2012-2005	PL2012-2010
1,000	1.09	PL2012-3001	PL2012-3005	PL2012-3010
1,300	1.07	PL2012-4001	PL2012-4005	PL2012-4010
2,000	1.05	PL2012-5001	PL2012-5005	PL2012-5010
3,000	1.04	PL2012-6001	PL2012-6005	PL2012-6010
5,000	1.03	PL2012-7001	PL2012-7005	PL2012-7010
7,000	1.04	PL2012-8001	PL2012-8005	PL2012-8010
10,000	1.02	PL2012-9001	PL2012-9005	PL2012-9010
20,000	1.02	PL2013-1001	PL2013-1005	PL2013-1010
30,000	1.02	PL2013-2001	PL2013-2005	PL2013-2010
50,000	1.03	PL2013-3001	PL2013-3005	PL2013-3010
70,000	1.03	PL2013-4001	PL2013-4005	PL2013-4010
100,000	1.02	PL2013-5001	PL2013-5005	PL2013-5010
130,000	1.01	PL2013-6001	PL2013-6005	PL2013-6010
200,000	1.05	PL2013-7001	PL2013-7005	PL2013-7010
300,000	1.03	PL2013-8001	PL2013-8005	PL2013-8010
500,000	1.03	PL2013-9001	PL2013-9005	PL2013-9010
700,000	1.03	PL2014-0001	PL2014-0005	PL2014-0010
1,000,000	1.05	PL2014-1001	PL2014-1005	PL2014-1010
1,500,000	1.04	PL2014-2001	PL2014-2005	PL2014-2010
2,000,000	1.04	PL2014-3001	PL2014-3005	PL2014-3010
2,500,000	1.05	PL2014-4001	PL2014-4005	PL2014-4010
4,000,000	1.04	PL2014-6001	PL2014-6005	PL2014-6010
7,000,000	1.04	PL2014-7001	PL2014-7005	PL2014-7010
10,000,000	1.06	PL2014-8001	PL2014-8005	PL2014-8010
15,000,000	1.06	PL2014-9001	PL2014-9005	PL2014-9010

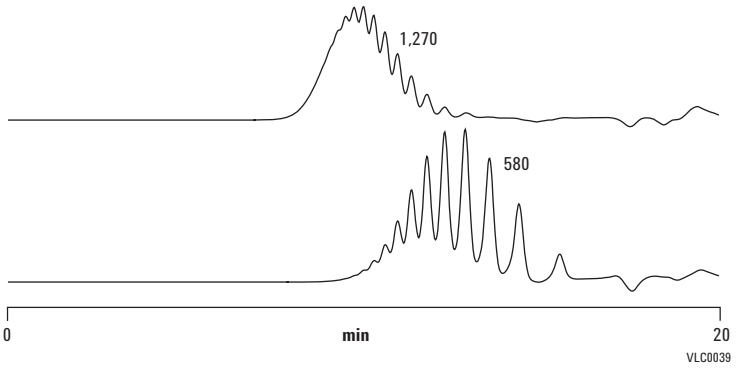
Polystyrene standards

Column: **2 x OligoPore PL1113-6520 7.5 x 300 mm**

Mobile Phase: THF

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



Polymethylmethacrylate

- Many solvent options increase applicability
- Stringent quality control improves performance
- Proprietary manufacturing methods ensure consistent supply

Polymethylmethacrylate (PMMA) standards are extremely versatile as they can be used for organic GPC with a wide range of medium polarity eluents, such as tetrahydrofuran, toluene, methyl ethyl ketone, and ethyl acetate. They also work well with more polar organic eluents, for example dimethylformamide, dimethylacetamide, and hexafluoroisopropanol. The MWs are selected to provide equidistant calibration points for greater accuracy, covering from 500-1.5 million MW. Every kit contains 0.5 g of ten different molecular weight standards.

Calibration Kits, (All Kits 10 x 0.5 g)

M-L-10 Part No. PL2010-0100	**M-M-10 Part No. PL2020-0101**
Constituent Polymer Nominal Mp (g/mol)	
600	1,000
840	2,200
1,400	5,000
2,350	11,200
3,900	25,500
6,400	58,000
10,800	130,000
18,000	290,000
30,000	660,000
50,000	1,500,000

Description Key

M: Medium
L: Low

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
500	1.19	PL2022-2001	PL2022-2005	PL2022-2010
1,000	1.26	PL2022-3001	PL2022-3005	PL2022-3010
2,000	1.08	PL2022-5001	PL2022-5005	PL2022-5010
3,000	1.08	PL2022-6001	PL2022-6005	PL2022-6010
5,000	1.09	PL2022-7001	PL2022-7005	PL2022-7010
7,000	1.08	PL2022-8001	PL2022-8005	PL2022-8010
10,000	1.03	PL2022-9001	PL2022-9005	PL2022-9010
13,000	1.03	PL2023-0001	PL2023-0005	PL2023-0010
20,000	1.03	PL2023-1001	PL2023-1005	PL2023-1010
30,000	1.02	PL2023-2001	PL2023-2005	PL2023-2010
50,000	1.02	PL2023-3001	PL2023-3005	PL2023-3010
70,000	1.02	PL2023-4001	PL2023-4005	PL2023-4010
100,000	1.02	PL2023-5001	PL2023-5005	PL2023-5010
130,000	1.05	PL2023-6001	PL2023-6005	PL2023-6010
200,000	1.02	PL2023-7001	PL2023-7005	PL2023-7010
300,000	1.02	PL2023-8001	PL2023-8005	PL2023-8010
500,000	1.06	PL2023-9001	PL2023-9005	PL2023-9010
700,000	1.03	PL2024-0001	PL2024-0005	PL2024-0010
1,000,000	1.09	PL2024-1001	PL2024-1005	PL2024-1010
1,500,000	1.09	PL2024-2001	PL2024-2005	PL2024-2010

Polymethylmethacrylate standards

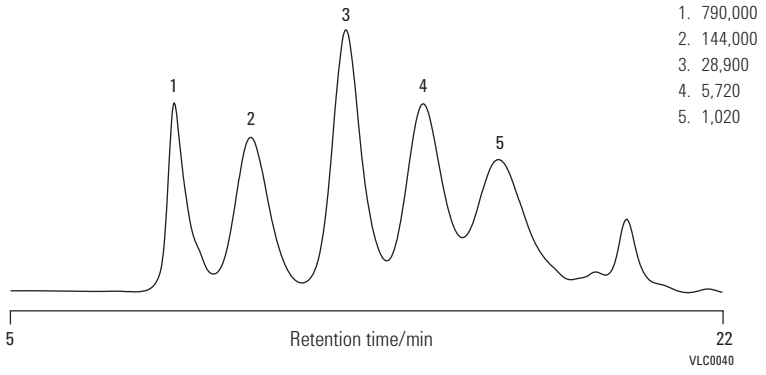
Column: **2 x PL HFIPgel PL1114-6900HFIP 7.5 x 300 mm**

Mobile Phase: HFIP + 20 mM NaTFAc

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: Agilent PL-GPC 50 (RI)



Polyethylene Glycol/Oxide

- Simple-to-use kit form
- Combines glycols and oxides to extend the MW range and cover more applications
- MWs selected to provide equidistant calibration points for greater accuracy

These hydrophilic polymers are suitable for both aqueous SEC and organic GPC using the majority of polar organic solvents. The oxides are available in high molecular weights, while the glycols cover the lower molecular weight range. The two types are chemically similar so they can be used together across a wider molecular weight range, with aqueous and organic polymers from 106-1 million MW. Every kit contains 0.2 g or 0.5 g of ten different molecular weight standards.

Calibration Kits

PEG-10 (10 x 0.5 g) **Part No.** **PL2070-0100**	**PEO-10 (10 x 0.2 g)** **Part No.** **PL2080-0101**
Constituent Polymer Nominal Mp (g/mol)	
106	20,000
194	30,000
400	50,000
600	70,000
1,000	100,000
2,000	200,000
4,000	300,000
7,000	400,000
13,000	700,000
20,000	1,000,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Nominal Mw/Mn	1 g Part No.	5 g Part No.	10 g Part No.
Polyethylene Glycol				
106	1.00	PL2070-1001	PL2070-1005	PL2070-1010
194	1.00	PL2070-2001	PL2070-2005	PL2070-2010
238	1.00	PL2071-2001	PL2071-2005	PL2071-2010
282	1.00	PL2071-3001	PL2071-3005	PL2071-3010
420	1.09	PL2070-3001	PL2070-3005	PL2070-3010
600	1.06	PL2070-4001	PL2070-4005	PL2070-4010
1,000	1.04	PL2070-5001	PL2070-5005	PL2070-5010
1,500	1.04	PL2070-6001	PL2070-6005	PL2070-6010
4,000	1.03	PL2070-7001	PL2070-7005	PL2070-7010
7,000	1.04	PL2070-8001	PL2070-8005	PL2070-8010
10,000	1.05	PL2070-9001	PL2070-9005	PL2070-9010
13,000	1.07	PL2071-0001	PL2071-0005	PL2071-0010
20,000	1.07	PL2071-1001	PL2071-1005	PL2071-1010
Polyethylene Oxide				
20,000	1.05	PL2083-1001	PL2083-1005	PL2083-1010
30,000	1.07	PL2083-2001	PL2083-2005	PL2083-2010
50,000	1.05	PL2083-3001	PL2083-3005	PL2083-3010
70,000	1.05	PL2083-4001	PL2083-4005	PL2083-4010
100,000	1.06	PL2083-5001	PL2083-5005	PL2083-5010
130,000	1.07	PL2083-6001	PL2083-6005	PL2083-6010
200,000	1.07	PL2083-7001	PL2083-7005	PL2083-7010
300,000	1.07	PL2083-8001	PL2083-8005	PL2083-8010
500,000	1.06	PL2083-9001	PL2083-9005	PL2083-9010
700,000	1.07	PL2084-0001	PL2084-0005	PL2084-0010
1,000,000	1.12	PL2084-1001	PL2084-1005	PL2084-1010
1,500,000	1.13	PL2084-2001	PL2084-2005	PL2084-2010

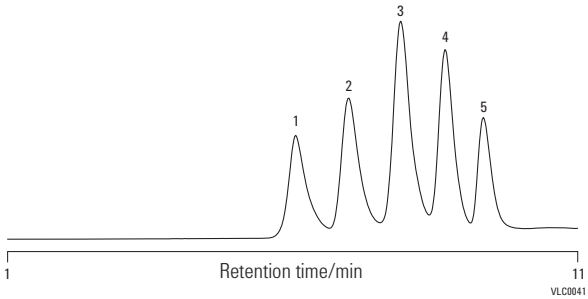
Polyethylene Glycol/Oxide standards

Column: **PL aquagel-OH MIXED-H 8 µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: Water

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



1. 1,702,000
2. 120,000
3. 12,600
4. 1,470
5. 106

Polysaccharides

- Comprehensive format provides full MW range in one handy kit
- Also available as individual standards

The pullulan polysaccharides kit consists of several simple sugars with relatively narrow polydispersity linear macromolecules of maltotriose units.

Calibration Kits

SAC-10 (10 x 0.2 g) **Part No.** **PL2090-0100**
Constituent Polymer Nominal Mp (g/mol)
180
738
5,000
10,000
20,000
50,000
100,000
200,000
400,000
700,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	Unit	Part No.
1,500	0.2 g	PL2091-2000
2,000	0.2 g	PL2091-3000
3,000	0.2 g	PL2091-4000
5,000	0.5 g	PL2090-1000
20,000	0.5 g	PL2090-3000
50,000	0.5 g	PL2090-4000
100,000	0.5 g	PL2090-5000
200,000	0.5 g	PL2090-6000
700,000	0.5 g	PL2090-8000
1,660,000	0.2 g	PL2091-1000

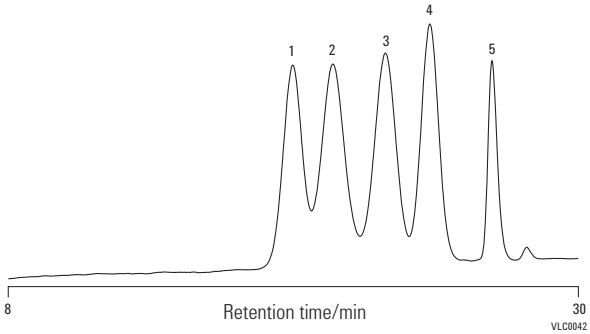
Pullulan polysaccharide standards

Column: **3 x PL aquagel-OH MIXED-H 8 µm**
PL1149-6800
7.5 x 300 mm

Mobile Phase: 0.2 M $NaNO_3$, 0.01 M NaH_2PO_4, pH 7

Flow Rate: 1.0 mL/min

Detector: Agilent PL-GPC 50 (RI)



1. 788,000
2. 212,000
3. 47,300
4. 11,800
5. 667

Polyacrylic Acid

- Compatible with all aqueous columns for wide applicability
- Aqueous polymers 1,000-2 million MW
- Well-characterized Mp values ensure wide utility

Calibration Kits

PAA-10 (10 x 0.2 g)
Part No.
PL2140-0100

Constituent Polymer Nominal Mp (g/mol)
1,000
3,000
7,000
13,000
30,000
70,000
100,000
300,000
700,000
1,000,000

Individual Polymer Molecular Weights

Polymer Nominal Mp (g/mol)	0.2 g Part No.	1 g Part No.
1,000	PL2142-3000	PL2142-3001
2,000	PL2142-5000	
3,000	PL2142-6000	PL2142-6001
5,000	PL2142-7000	PL2142-7001
7,000	PL2142-8000	PL2142-8001
13,000	PL2143-0000	PL2143-0101
30,000	PL2143-2000	PL2143-2001
50,000	PL2143-3000	PL2143-3001
70,000	PL2143-4000	PL2143-4001
100,000	PL2143-5000	PL2143-5001
130,000	PL2143-6000	PL2143-6001
200,000	PL2143-7000	PL2143-7001
300,000	PL2143-8000	PL2143-8001
500,000	PL2143-9000	PL2143-9001
700,000	PL2144-0000	PL2144-0101
1,000,000	PL2144-1000	PL2144-1001
1,500,000	PL2144-2000	PL2144-2001
2,000,000	PL2144-3000	PL2144-3001

LC and LC/MS Troubleshooting

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Baseline disturbance at void time	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
Detector leaks	Plugged inlet frit	Replace seals/gaskets
Drifting baseline	Positive direction – Contaminant buildup/elution	Flush column, clean up sample, use pure solvents
	Positive/negative – Difference in refractive index of injection solvent	Use mobile phase for sample solvent
	Negative direction (gradient) – Absorbance of "A" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Positive direction (gradient) – Absorbance of "B" mobile phase solvent	Use non-absorbing or HPLC-grade or better solvent
	Random – Temperature changes	Insulate column and tubing
	Random – Temperature changes	Thermostat column and tubing
	Wavy or undulating – Temperature changes in room	Monitor room temperature and control
Ghost peaks	Peaks from previous injection	Flush column to remove contaminants
	Contamination	Sample cleanup or pre-fractionation
	Unknown interferences in samples	Sample cleanup or pre-fractionation
	Ion-pair – Upset equilibrium	Prepare sample in actual mobile phase to minimize disturbance
	Peptide mapping – Oxidation of TFA	Prepare fresh daily; use anti-oxidant
	Reversed-phase – Contaminated water	Check suitability of water by running different amount through reversed-phase column and measure peak height with elution; use HPLC grade solvents
	Spikes – Bubbles in solvent	De-gas solvents
High column backpressure	Column blockage, adsorbed sample	Better sample cleanup; use guard column
	Mobile phase viscosity too high	Use lower viscosity solvents or higher temperature
	Particle size too small	Use larger d_p packing
	Plugged inlet frit	Replace column
	Plugged inlet frit	Reverse solvent flow
Leaks	Subtle – White powder at fitting/loose fitting	Tighten fittings, cut tubing, or replace ferrules
Leaks, injection valve	Catastrophic – Worn valve rotor	Replace rotor in valve
Leaks, column or other fittings	Catastrophic – Loose fittings	Tighten or replace fittings
Leak, pump	Catastrophic – Pump seal failure	Replace pump seal

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Negative peaks	RI detector – solute refractive index less than solvent	No problem; reverse polarity to make positive
	UV detector – solute absorbance less than mobile phase	Use mobile phase with lower UV absorbance; do not recycle solvent too long
Noisy baseline	Random – Contaminant buildup	Flush column; clean up sample; use HPLC-grade solvent
	Continuous – Detector lamp problem	Replace detector lamp
	Occasional – External electrical interference	Use voltage stabilizer for LC system
Peak doubling	Sample volume too large	Reduce the volume e.g. by half and re-inject
	Injection solvent too strong	Use weaker injection solvent or mobile phase
	Blocked frit	Replace and use 0.5 µm porosity in-line filter
	Column void or channeling	Replace column; for some columns, fill in void with packing
	Unswept injector flowpath	Replace injector rotor
	Void at head of column	Replace column, top off column with packing
	Column overloaded with sample	Use higher capacity stationary phase Increase column diameter Decrease sample size
	Single peak – interfering components	Sample cleanup; pre-fractionation
Peak tailing	Beginning of peak doubling	See "peak doubling"
	Unswept dead volumes	Minimize number of connections Ensure injector seal is tight Ensure fittings are properly seated
	Basic compounds – Silanol interactions	Choose endcapped bonded phase Switch to polymeric phase
	Basic substances – Silanol interactions	Use stronger mobile phase or add competing base (e.g. TMA)
	Silica-based – Column degradation	Use specialty column; polymeric column or sterically protected

(Continued)

HPLC Troubleshooting

Symptom Type	Possible Cause	Solution
Peaks are broad	Injection volume too large	Decrease solvent strength of injection solvent to focus solute
	Peak dispersion in injector valve	Introduce air bubble in front/back of sample to decrease dispersion
	Sampling rate of data system too slow	Increase frequency of sampling
	Slow detector time constant	Adjust time constant to match peak width
	Mobile phase viscosity too high	Increase column temperature
	Detector cell volume too large	Use smallest possible cell volume with no heat exchanger in system
	Injector volume too large	Decrease injection volume
	Long retention times	Use gradient elution or stronger mobile phase
Pressure fluctuation	Leaky check valve	Replace check valve
	Pump seal leaks	Replace pump seals
	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
Pressure increasing	Buildup of particulates	Filter sample; in-line filter; filter mobile phase
	Water/organic systems – buffer precipitation	Test buffer-organic mixtures; ensure compatibility
Retention beyond total permeation volume	Size exclusion – Specific interactions	Add mobile phase modifiers or change solvent
Retention times changing	Column temperature varying	Thermostat column; insulate column; ensure lab temperature constant
	Equilibration time insufficient with gradient run or changes in isocratic mobile phase	Make sure at least 10 column volumes pass through column after solvent change or gradient conclusion
	Selective evaporation of mobile phase component	Less vigorous helium sparging; keep solvent reservoirs covered; prepare fresh mobile phase
	Buffer capacity insufficient	Use >20 mM concentration of buffer
	Inconsistent on-line mobile phase mixing	Ensure gradient system delivering constant composition; check vs. manual prep of mobile phase
	Contamination buildup	Occasionally flush column with strong solvent to remove contaminants
	First few injections – Adsorption on active sites	Condition column by initial injection of concentrated sample

(Continued)

HPLC Troubleshooting		
Symptom Type	**Possible Cause**	**Solution**
Retention times decreasing	Flow rate increasing	Check pump to make sure correct; if not, reset
	Column overloaded with sample	Decrease sample size
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
Retention times increasing	Flow rate is slowing	Fix leaks in liquid lines, replace pump seals, check for pump cavitation or air bubbles
	Active sites on silica packing	Use mobile phase modifier
	Loss of bonded stationary phase	Keep mobile phase pH between 2 and 8.5
	Mobile phase composition changing	Make sure mobile phase container is covered
	Active sites on silica packing	Add competing base to mobile phase
	Active sites on silica packing	Use higher coverage packing for stationary phase
Sensitivity problem	Peaks are outside of linear range of detector	Dilute/concentrate to bring into linear region
	First few sample injections – Absorption of sample in loop or column	Condition loop/column with concentrated sample
	Autosampler flow lines blocked	Check flow and make sure there are no blockages
	Injector sample loop underfilled	Make sure that loop is overfilled with sample
	Sample-related losses during preparation	Use internal standard during sample prep; optimize sample prep method
Slow column equilibration times (ion-pairing)	Equilibration time slow for long-chain ion-pairing reagents	Use shorter alkyl chain ion-pair reagent

LC/MS Troubleshooting

Symptom Type	Solution
No peaks	Spray from the nebulizer
	Make sure capillary voltage is set correctly
	Make sure LC/MSD is tuned correctly
	Make sure LC/MSD pressures are within normal ranges
	Check drying gas flow and temperature
	Make sure fragmentor is set correctly
Poor mass accuracy	Recalibrate the mass axis
	Make sure ions used for tuning span mass range of sample ions and show strong stable signals
Low signal	Check the solution chemistry; make sure solvent is appropriate for sample
	Make sure sample is fresh and has been stored correctly
	Make sure LC/MSD is tuned correctly
	Check the nebulizer condition
	Clean the capillary entrance
	Check the capillary for damage and contamination
Unstable signal	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow

(Continued)

LC/MS Troubleshooting

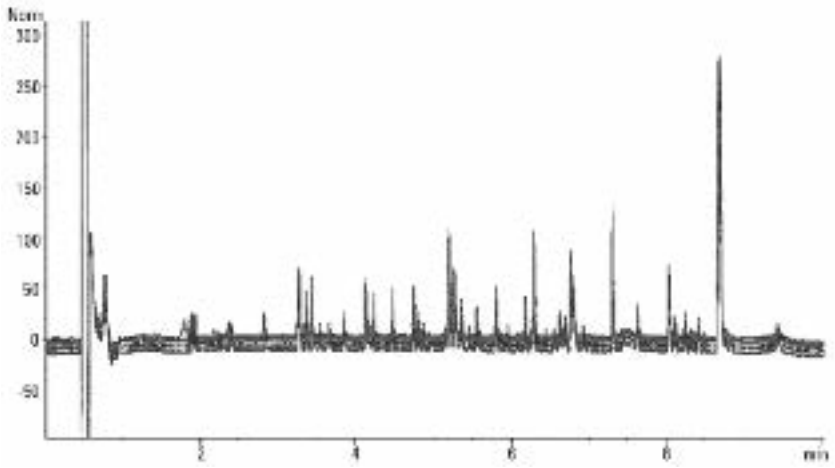
Symptom Type	Solution
High spectral noise	Use appropriate mass filter values
	Check spray shape; nebulizer may be damaged or set incorrectly
	Make sure drying gas flow and temperature are correct for the solvent flow
	Make sure solvent is throroughly degassed
	Make sure LC backpressure is steady; this indicates a steady solvent flow
	If you are using water as part of the mobile phase, make sure it is de-ionized (> 18 MΩ cm)
Droplets, not spray, exiting the nebulizer	Make sure nebulizing gas pressure is set high enough for the LC flow
	Check position of needle in nebulizer
	Stop solvent flow and remove nebulizer assembly
	Examine end of nebulizer for damage
No flow	Make sure LC is on and there is sufficient solvent in correct bottle
	Check for LC error messages
	Check for blockages
	Repair or replace any blocked components
	Check for leaks
	Make sure MS stream selector valve is set to LC to MSD
Undesired fragmentation	(APCI vs. Electrospray)
	APCI temperature is too high
	Fragmentor voltage is set too high

BioPharmaceutical Applications

NEW!

Protein digest analysis

Column:	**ZORBAX 300SB-C18 858750-902 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.085% TFA in ACN
Flow Rate:	0.5 mL/min
Pressure:	640 bar
Gradient:	2% B 1 min, 2-45% B 8.8 min, 45-95% B 0.2 min, 95% B 2 min, 98-2% B 0.2 min, 2% B 1.8 min
Temperature:	50 °C
Detector:	Agilent 1290 Infinity LC
Injection:	5 µL
Sample:	Protein digest
Sample Conc:	1 mg/mL

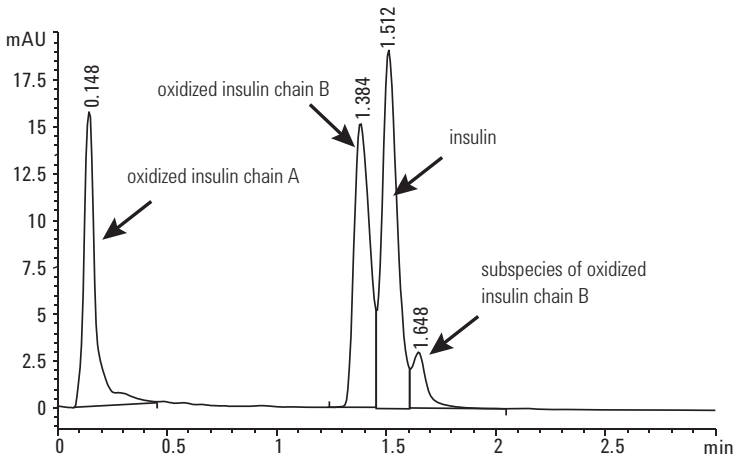


Overlaid chromatograms of 30 runs of a protein digest on an Agilent ZORBAX RRHD 300SB-C18 column.

NEW!

Analysis of oxidized insulin chains

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% ACN + 0.01% TFA in water
Flow Rate:	1.0 mL/min
Pressure:	650-700 bar
Gradient:	33-50% B, 0-4 min; 33% B, 4-5 min
Detector:	UV, 280 nm Agilent 1290 Infinity LC
Sample:	Insulin, oxidized insulin chain A and chain B from bovine pancreas (Sigma Aldrich, St. Louis, MO)
Sample Conc:	1 mg/mL
Injection:	2 µL

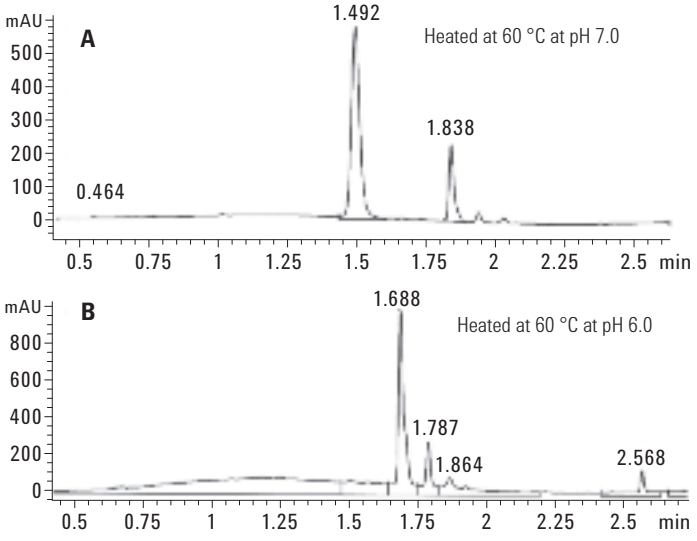


Insulin and oxidized insulin A and B chains are resolved quickly but insulin and oxidized chain B often co-elute.

NEW!

Fast separation of recombinant human erythropoietin

Column:	**ZORBAX RRHD 300SB-C18 857750-902 2.1 x 50 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 0.01% TFA in ACN
Flow Rate:	1.0 mL/min
Pressure:	650 bar
Gradient:	5 to 100% B solvent from 0 to 2.5 min
Detector:	UV, 280 nm Agilent 1290 Infinty LC
Sample:	Recombinant human EPO protein (rEPO)
Sample Conc:	1.0 mg/mL
Injection:	3 µL



Heat-treated rEPO protein are well resolved by the Agilent ZORBAX RRHD 300SB-C18 column. The column separated these heat-treated rEPO proteins.

NEW!

Separation optimization for ultra fast analysis of reduced and alkylated monoclonal antibody

Column: **ZORBAX RRHD 300SB-C8**
858750-906
2.1 x 100 mm, 1.8 µm

Mobile Phase: (Various)
A: H_2O + 0.1% TFA (v/v)
B: n-propanol:ACN:H_2O (80:10:10) + 0.1% TFA (v/v)

Injection: 1-3 µL

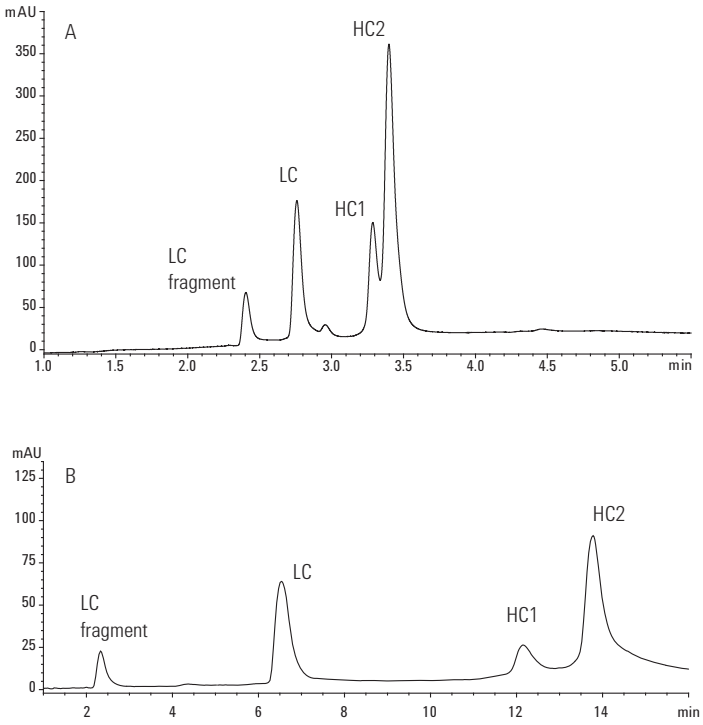
Flow Rate: 0.5 mL/min

Gradient: Multi-segmented
A (optimized for speed): 0 min-20% B, 3 min-35% B, 4 min-40% B, 5 min-40% B, 5.1 min-90% B, 5.5 min-90% B, 6 min-25% B
B (optimized for resolution): 0 min-25% B, 15 min-32% B, 16 min-32% B, 17 min-90% B, 17.5 min-90% B, 18 min-25% B

Temperature: 75 °C

Detector: UV, 225 nm
Agilent 1290 Infinity LC

For consecutive chromatographic runs, a 2-minute post run was added to re-equilibrate the column.



Comparison of two optmimized gradients for the ultra fast separation of reduced and alkylated monoclonal antibodies on an Agilent ZORBAX RRHD 300SB-C8 column. The top panel details a rapid separation of the light and heavy chain variants in a shortened run time of less than 4 minutes. The bottom panel displays complete baseline resolution of the two heavy chain variants during a longer runtime using a shallower gradient profile. Both separations were performed at 75 °C and completed with a fast 90% 1-propanol wash step (UV not shown).

NEW!

Column reproducibility – 200 injections of reduced monoclonal antibody using an Agilent ZORBAX RRHD 300SB-C3 column

Column: **Agilent ZORBAX RRHD 300SB-C3**
858750-909
2.1 x 100 mm, 1.8 µm

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

Flow Rate: 0.4 mL/min

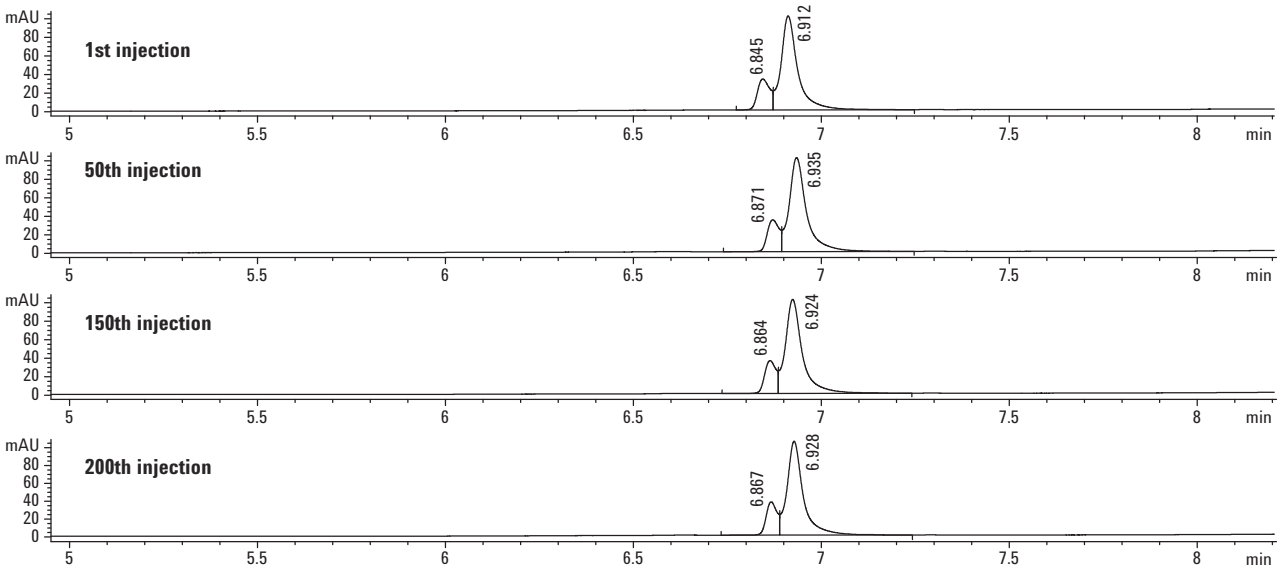
Gradient: 0 min-1% B, 2 min-20% B, 5 min-50% B, 7 min-50% B, 8.0 min-90% B, 8.3 min-1% hold for 2 min

Temperature: 75 °C

Detector: UV, 280
Agilent 1290 Infinity LC

Sample: Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - Agilent BL05 IgG1

Injection: 2 µL



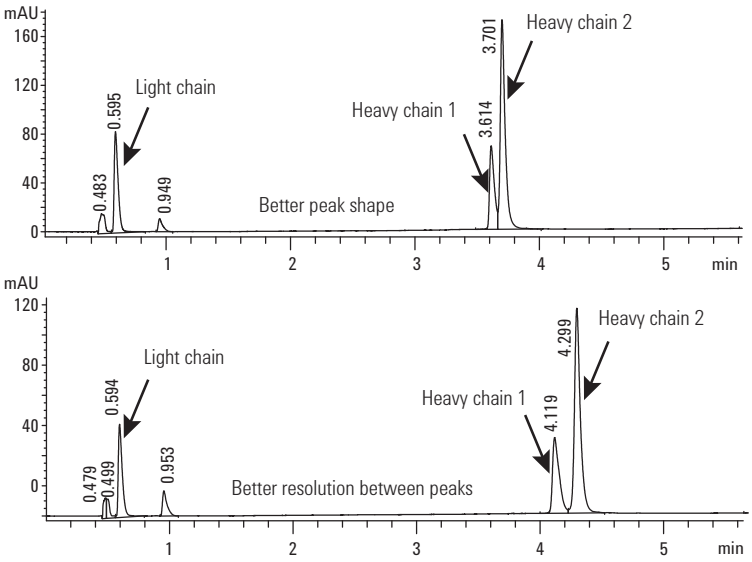
Reduced and alkylated mAb profiling during 200 repeated injections.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Gradient optimizations for ultra fast analysis of reduced monoclonal antibody

Column:	**Agilent ZORBAX RRHD 300SB-Diphenyl 858750-944 2.1 x 100 mm, 1.8 µm**
Mobile Phase:	A: 0.1% TFA in water B: 80% propyl alcohol, 10% ACN, 9.9% water and 0.1% TFA
Flow Rate:	0.5 mL/min
Gradient:	1st condition: 0 min-1% B, 2 min-20% B, 5 min-70% B 2nd condition: 0 min-1% B, 2 min-20% B, 5 min-50% B
Temperature:	74 °C
Detector:	UV, 280 nm
Sample:	Reduced monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1
Injection:	2 µL



Comparison of two ultra-fast separations of reduced monoclonal antibodies was achieved on a Agilent ZORBAX RRHD 300SB-Diphenyl under different optimized conditions. The top panel separation delivered narrow peak widths with shorter retention times. The bottom panel separation displays higher resolution between the two heavy chain peaks, but with less efficiency.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Ultra high speed and high resolution of intact monoclonal antibodies

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

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

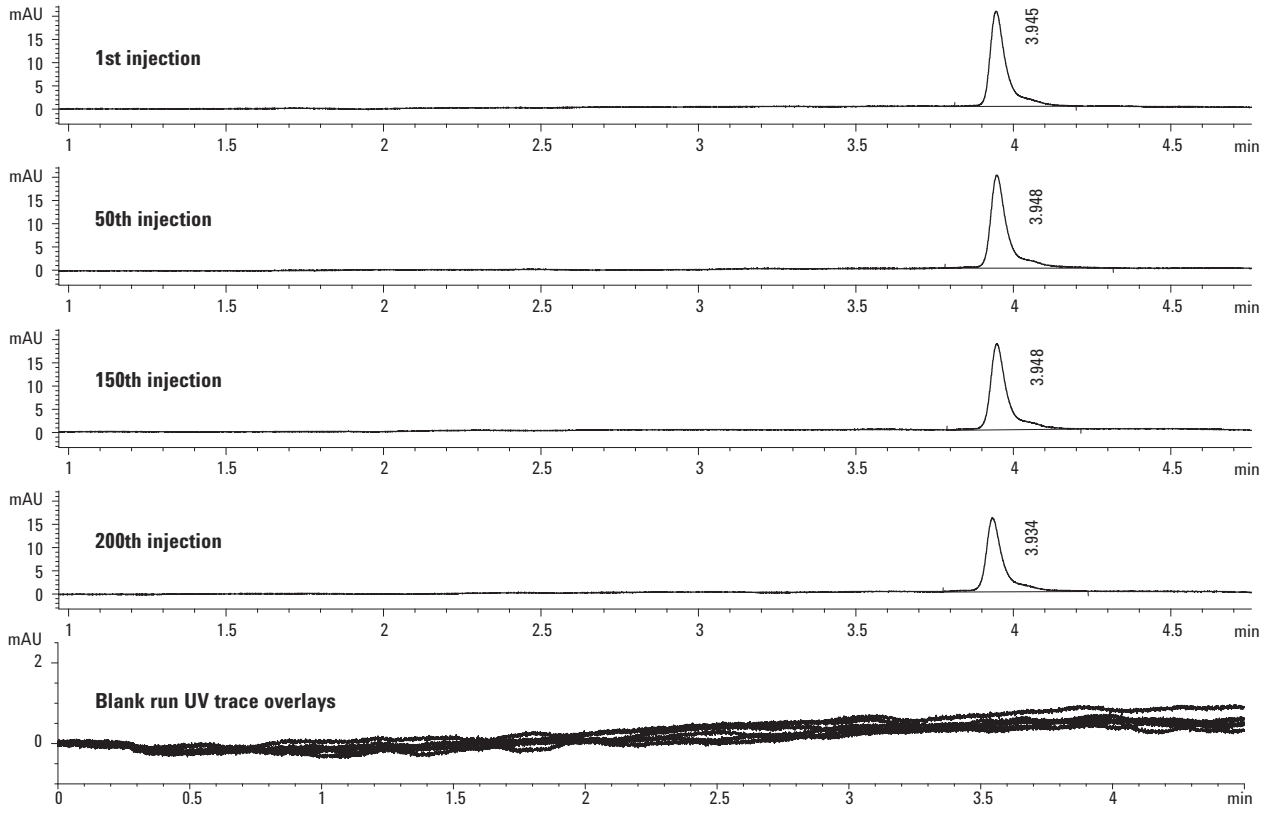
Flow Rate: 1.0 mL/min

Temperature: 74 °C

Detector: UV, 280 nm

Sample: Monoclonal antibody (IgG1) (1.0 mg/mL) - BioCreative IgG1 and Agilent Standard IgG1

Injection: 1 µL



Details of intact mAb profiling during 200 repeated injections. Intact mAb separations shown were collected at 1, 50, 150, and 200th run intervals. The bottom panel displays 5 UV blank run trace overlays collected every 20th run during the column evaluation (**note:** overlay traces are scaled to 2 mAu).

NEW!

Optimizing protein separations with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 40.0 mM NaH_2PO_4
D: 40.0 mM Na_2HPO_4
By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)

Flow Rate: 1.0 mL/min

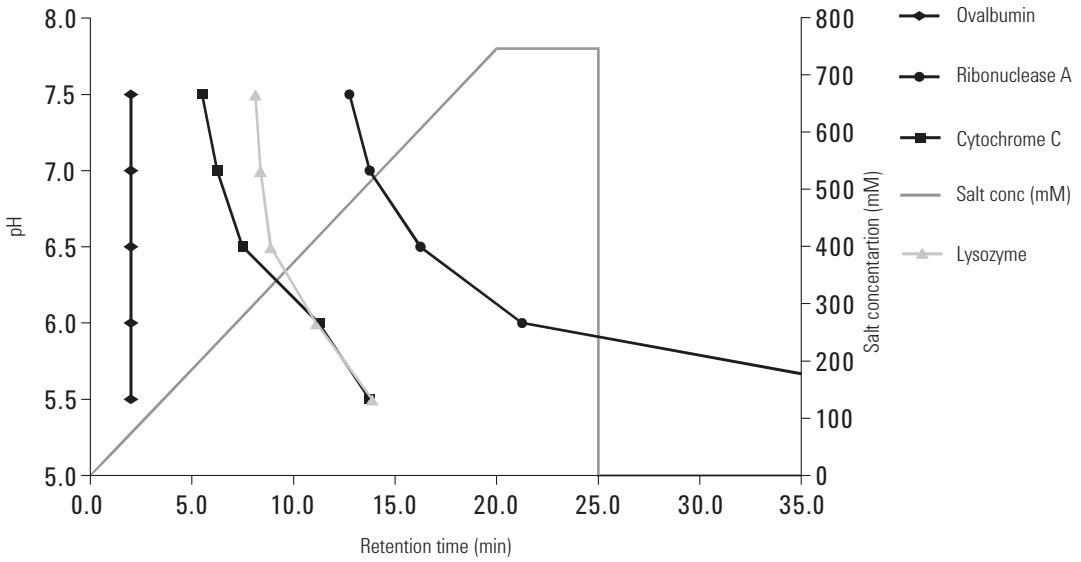
Gradient: 0 to 50% B, 0 to 20 min
50% B, 20 to 25 min
0% B, 25 to 35 min

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Effect of pH on retention time of protein standards using a Agilent Bio WCX column.

NEW!

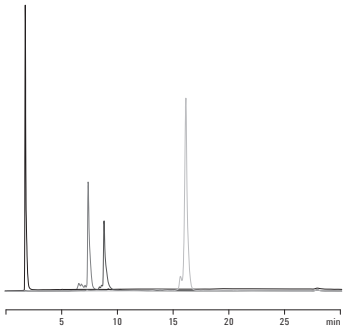
Improved resolution with smaller particle size with Agilent weak cation-exchange columns

Column: **Agilent Bio WCX, stainless steel**
5190-2453
4.6 x 250 mm, 10 µm

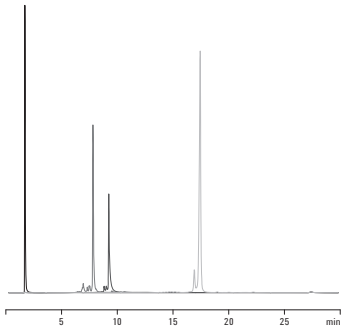
Column: **Agilent Bio WCX, stainless steel**
5190-2445
4.6 x 250 mm, 5 µm

Mobile Phase:	A: water B: 1.6 M NaCl C: 40.0 mM NaH_2PO_4 D: 40.0 mM Na_2HPO_4 By combining predetermined proportions of C and D, 20 mM buffer solutions at the desired pH range were produced (proportions determined using Buffer Advisor software)
Gradient:	0 to 50% B, 0 to 20 min 50% B, 20 to 25 min 0% B, 25 to 35 min
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme



Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP10 column.

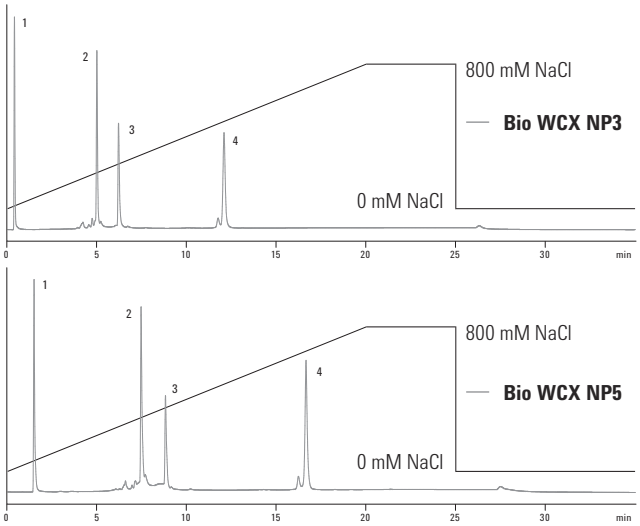


Separation of protein standards at pH 6.5 using an Agilent Bio WCX, NP5 column.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

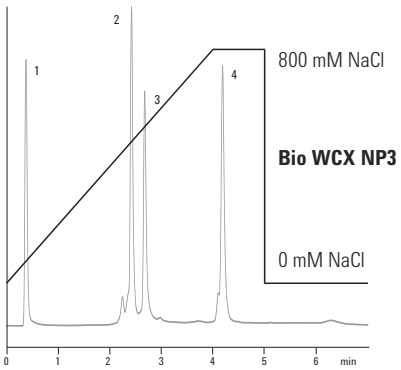
Faster separations using Agilent weak cation-exchange columns

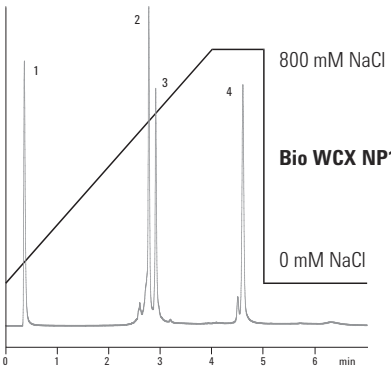


Protein separation on Agilent Bio WCX NP5 versus Agilent Bio WCX NP3.

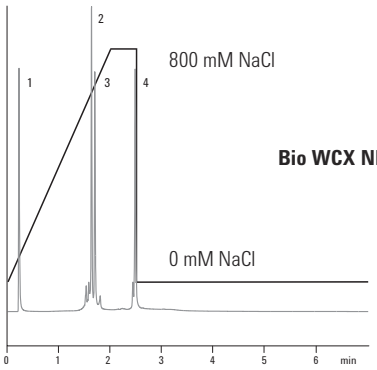
Column:	**Agilent Bio WCX, stainless steel 5190-2445 4.6 x 250 mm, 5 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2443 4.6 x 50 mm, 3 µm**
Column:	**Agilent Bio WCX, stainless steel 5190-2441 4.6 x 50 mm, 1.7 µm**
Mobile Phase:	A: 20 mM sodium phosphate, pH 6.5 B: A + 1.6 M NaCl
Flow Rate:	1.0 mL/min
Gradient:	0 to 50% B
Temperature:	Ambient
Detector:	UV, 220 nm Agilent 1260 Infinity Bio-inert Quaternary LC
Sample:	Ovalbumin, Ribonuclease A, Cytochrome c, Lysozyme
Sample Conc:	0.5 mg/mL

1. Ovalbumin
2. Ribonuclease A
3. Cytochrome c
4. Lysozyme





Comparison of Agilent Bio WCX NP3 versus Agilent Bio WCX NP1.7 (flow rate 1.0 mL/min).



Agilent Bio WCX NP1.7 for protein separations under 3 minutes (flow rate 1.7 mL/min).

NEW!

pH gradient elution for improved separation of monoclonal antibody charged variants

Column: **Bio MAb, stainless steel**
5190-2405
4.6 x 250 mm, 5 µm

Mobile Phase: A: water
B: 1.6 M NaCl
C: 100 mM NaH_2PO_4
D: 100 mM Na_2HPO_4
By combining predetermined proportions of C and D, buffer solutions at the desired pH range were produced at the selected buffer strengths.

Flow Rate: 1.0 mL/min

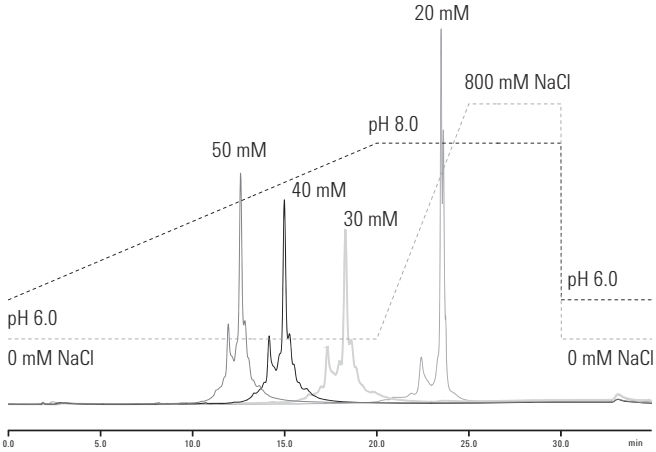
Gradient: pH 6.0 to 8.0, 0 to 20 minutes
0 to 800 mM NaCl, 20 to 25 minutes
800 mM NaCl, 25 to 30 minutes

Temperature: Ambient

Detector: UV, 220 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: IgG monoclonal antibody

Sample Conc: 2 mg/mL (in 20 mM sodium phosphate buffer, pH 6.0)



Chromatograms of IgG monoclonal antibody at different ionic strengths.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Separation of recombinant human erythropoietin (rEPO) using Agilent Bio SEC-3

Column: **Bio SEC-3, 100Å**
5190-2503
4.6 x 300 mm, 3 µm

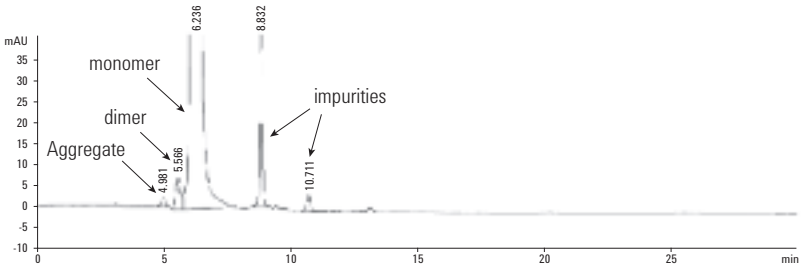
Mobile Phase: 150 mM sodium phosphate buffer, pH 7.0

Flow Rate: 0.35 mL/min

Detector: UV, 225 nm
Agilent 1260 Infinity Bio-inert Quaternary LC

Sample: Recombinant human EPO protein (rEPO)

Sample Conc: 1.0 mg/mL



Consistent ion-exchange MAb separation

Column: **Bio MAb, PEEK**
5190-2411
2.1 x 250 mm, 5 µm

Buffer: A: Sodium phosphate buffer, 20 mM
B: Buffer A + 400 mM NaCl

Gradient: 15-35% Buffer B from 0-30 min

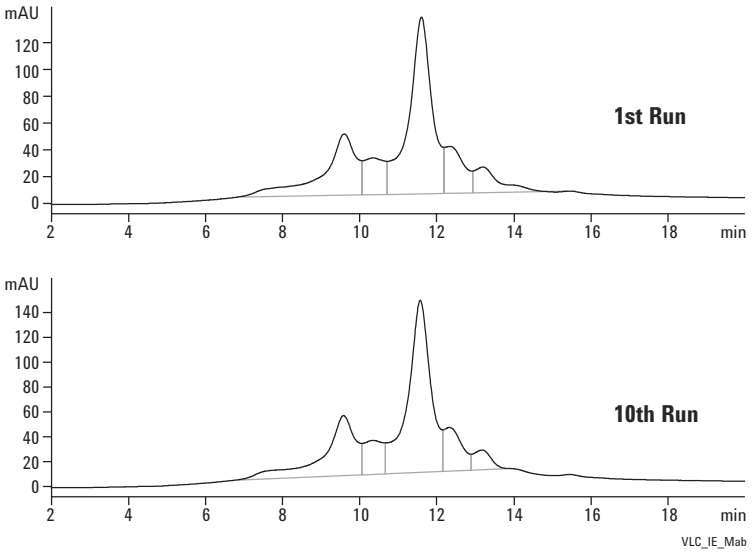
Flow Rate: 0.65 mL/min

Sample: CHO-humanized MAb, 1 mg/mL

Injection: 2.5 µL

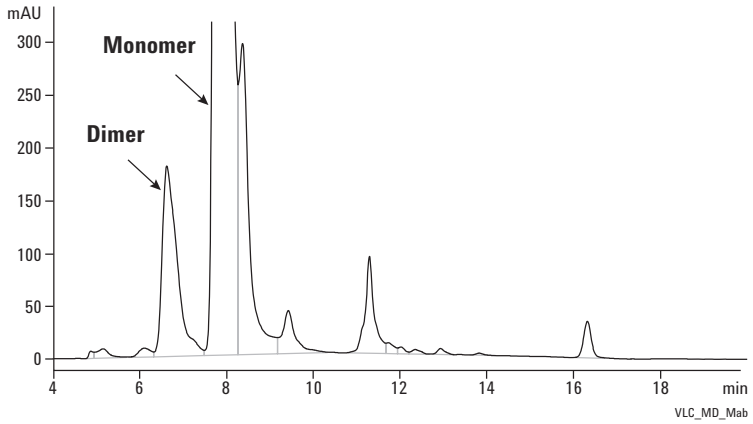
Detector: UV, 220 nm

Temperature: Ambient



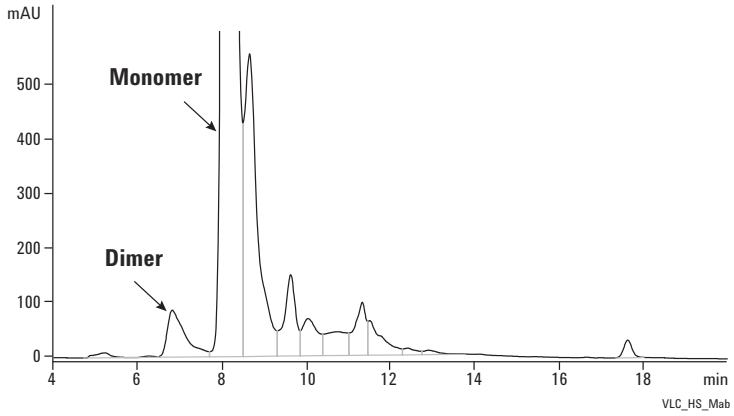
Intact MAb monomer and dimer separation

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



Separation of heated, stressed MAb

Column:	**Bio SEC-3, 300Å 5190-2511 7.8 x 300 mm, 3 µm**
Buffer:	Sodium phosphate buffer, pH 7.0, 150 mM +150 mM sodium sulfate
Gradient:	0-100% Buffer A from 0-30 min
Flow Rate:	1.0 mL/min
Sample:	CHO-humanized MAb, 5 mg/mL – stressed at 60 °C
Injection:	5 µL
Detector:	UV, 220 nm
Temperature:	Ambient



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Nucleosides, purines and pyrimidines

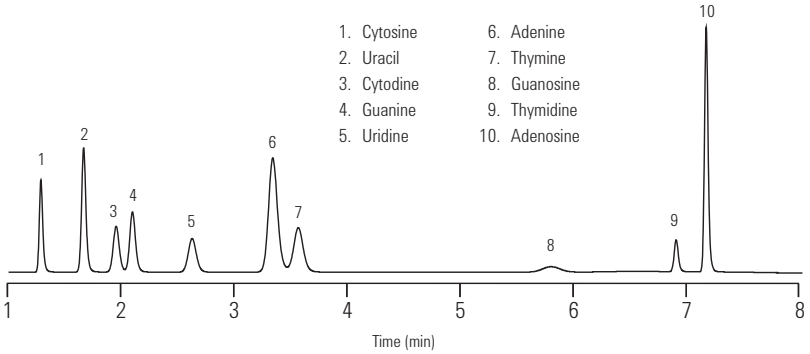
Column: **Eclipse Plus Phenyl Hexyl**
959993-912
4.6 x 150 mm, 5 µm

Mobile Phase: 1% MeOH: 99% 20 mm Ammonium Acetate, pH 4.5

Flow Rate: 1 mL/min

Detector: UV, 254 nm

1. Cytosine
2. Uracil
3. Cytodine
4. Guanine
5. Uridine
6. Adenine
7. Thymine
8. Guanosine
9. Thymidine
10. Adenosine



nucleosides

Amino acid standard separation on Eclipse Plus C18

Column: **Eclipse Plus C18**
959763-902
2.1 x 150 mm, 3.5 µm

Mobile Phase: A: 10 mM Na_2HPO_4, 10 mM $Na_2B_4O_7$, 0.5 mM NaN_3, pH 8.2
B: acetonitrile: methanol: water (45:45:10) (v/v/v)

Flow Rate: 0.42 mL/min

Temperature: 40 °C

Detector: UV, 338 nm, then switch to 280 nm at 15.7 min

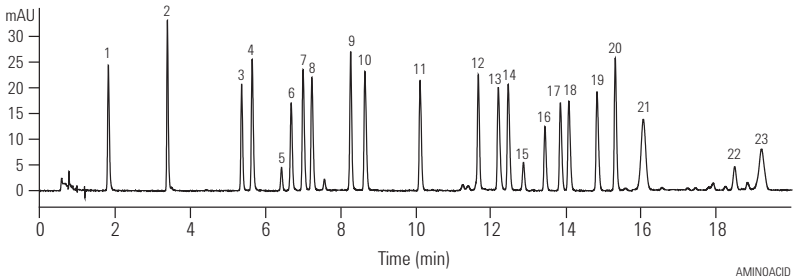
Sample: 900 pmol Amino Acids with extended Amino Acids and Internal Standards (500 pmol)

Derivatization: Automated, online, OPA / FMOC

1. ASP
2. GLU
3. ASN
4. SER
5. GLN
6. HIS
7. GLY
8. THR
9. ARG
10. ALA
11. TYR
12. CY2
13. VAL
14. MET
15. NVA
16. TRP
17. PHE
18. ILE
19. LEU
20. LYS
21. HYP
22. SAR
23. PRO

Gradient

Time (min)	% B
0	2
0.5	2
20	57
20.1	100
23.5	100
23.6	2
25	stop



AMINOACID

Antibodies: Fast separation of IgM and IgG antibodies

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

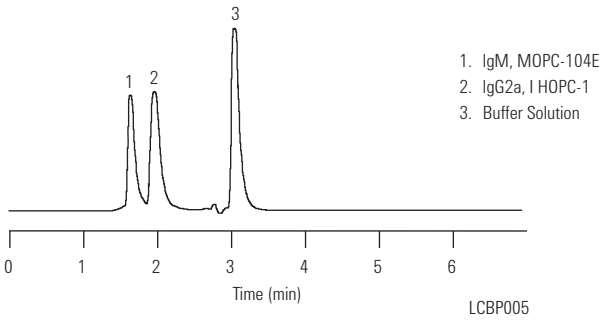
Mobile Phase: 200 mM Sodium Phosphate (pH 7), 0.01% Azide

Flow Rate: 0.94 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 2.5 µL (1 mg/mL)



Glycosylated proteins: Large molecules on Poroshell 300SB-C18 and 300SB-C8

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

Column B: **Poroshell 300SB-C8**
661750-906
1.0 x 75 mm, 5 µm

Column C: **ZORBAX 300SB-C18**
865630-902
1.0 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.07% TFA in ACN

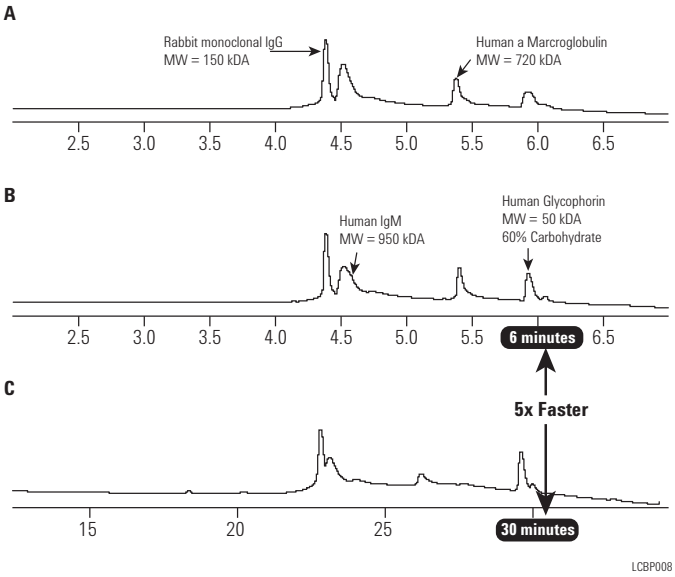
Flow Rate: A, B: 0.454 mL/min
C: 0.071 mL/min

Gradient: A, B: 0 min 5% B
10 min 100% B
C: 0 min 5% B
50 min 100% B

Temperature: 70 °C

Detector: DAD 212 nm, 1.7 µL flow cell, <0.01 min peak width

Sample: Large glycosylated proteins



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

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

HSA tryptic digest on ZORBAX Rapid Resolution HT 1.8 µm

Column A: **ZORBAX SB-C18**
883700-922
2.1 x 150 mm, 5 µm

Column B: **ZORBAX SB-C18**
822700-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: Water w/0.1% TFA
B: ACN w/0.1% TFA

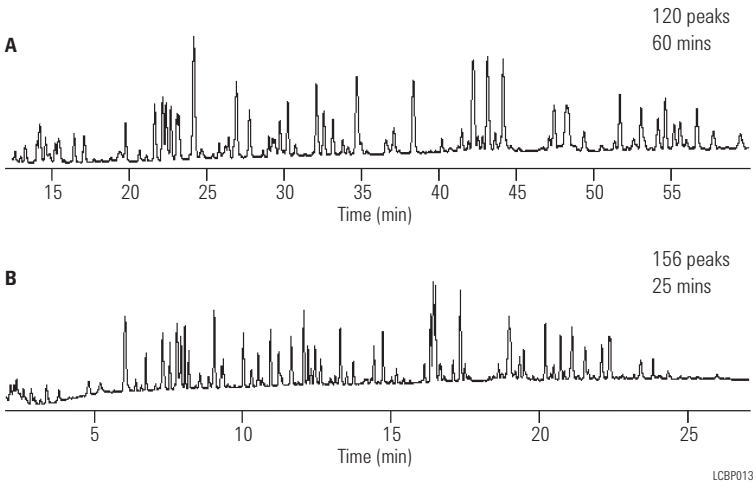
Flow Rate: A: 0.2 mL/min
B: 0.5mL/min

Gradient: A: 2 to 50% B in 70min
B: 2 to 50% B in 30min

Temperature: 50 °C

Detector: UV, 214 nm

Sample: HSA tryptic digest, 8 µL of 15 pmol/µL (120 pmol on column)



Human serum: Low abundance protein isolation and identification from 1-D gel band by LC/MS

Column: **ZORBAX 300SB-C18**
Trap: 0.3 x 5 mm, 5 µm, 5065-9913
Analytical: 0.3 x 150 mm,
5 µm, 5064-8263

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

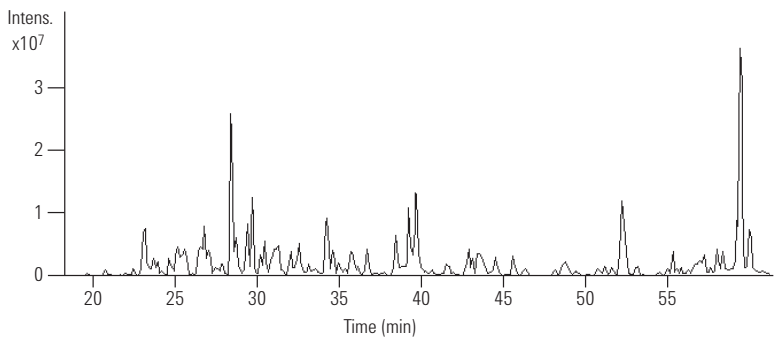
Flow Rate: 6 µL/min

Gradient: 0 min 3% B
5 min 3% B (loading)
50 min 45% B
52 min 80% B
57 min 80% B
60 min 3% B

Sample: Band from 1-D in gel digest

Sample Preparation of Human Serum:
Major serum proteins removed using Multiple Affinity Removal
Column: 4.6 x 100 mm, P/N 5185-5985
Followed by 1-D gel digest

Base Peak Chromatogram



Proteins Identified

1. α-1-Antichymotrypsin
2. Antithrombin-III Precursor
3. Complement Factor B Precursor

Monoclonal IgG1 chains: Separation on Poroshell 300SB-C8

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

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

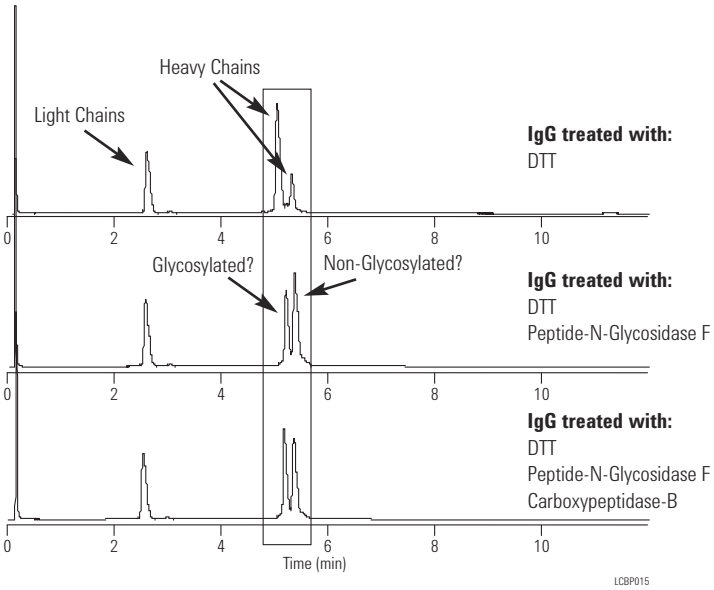
Flow Rate: 1.0 mL/min

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

Temperature: 70 °C

Sample: Monoclonal IgG1

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



Use ZORBAX Extend-C18 for alternate selectivity at high pH

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

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

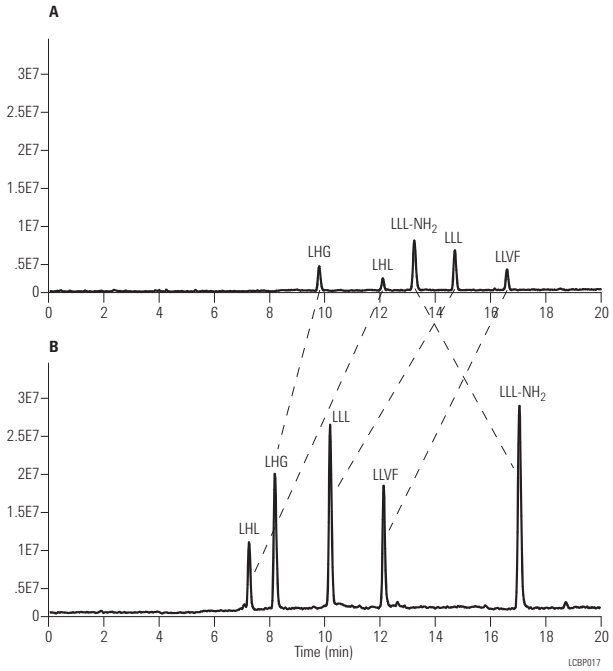
A: 20 mM NH_4OH in Water
B: 20 mM NH_4OH in 80% ACN

Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

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



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

Nucleosides: Separation of deoxy and ribonucleosides

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

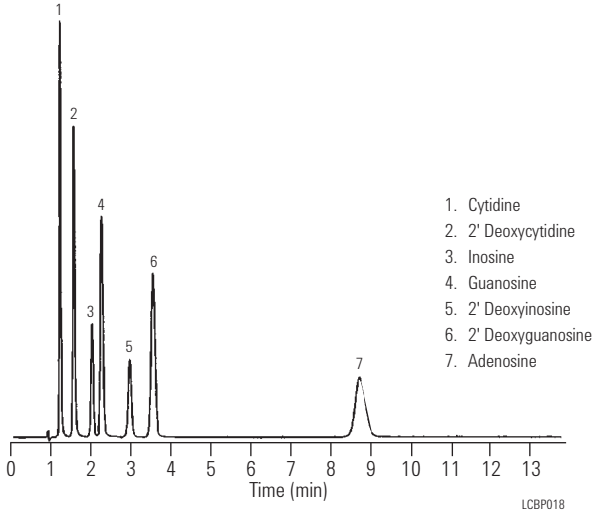
Mobile Phase: 4 mM Ammonium Phosphate (pH 4.0 with Phosphoric Acid)

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL (1.6 µg each)



1. Cytidine
2. 2' Deoxycytidine
3. Inosine
4. Guanosine
5. 2' Deoxyinosine
6. 2' Deoxyguanosine
7. Adenosine

Nucleotides: Separation of mononucleotides

Column: **ZORBAX SAX**
880952-703
4.6 x 250 mm, 5 µm

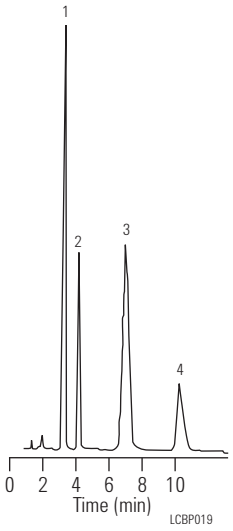
Mobile Phase: 0.1 M $NH_4H_2PO_4$

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Orotic Acid, UMP, GMP, XMP



1. Orotic Acid
2. UMP
3. GMP
4. XMP

Separation of basic peptides on Bonus-RP versus traditional Alkyl phase

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

Column B: **Alkyl C8**

Mobile Phase: A: 0.010 M ammonium phosphate, pH 7/0.050 M sodium perchlorate
B: 0.010 M ammonium phosphate/0.050 M sodium perchlorate in 50% ACN

Flow Rate: 1.0 mL/min

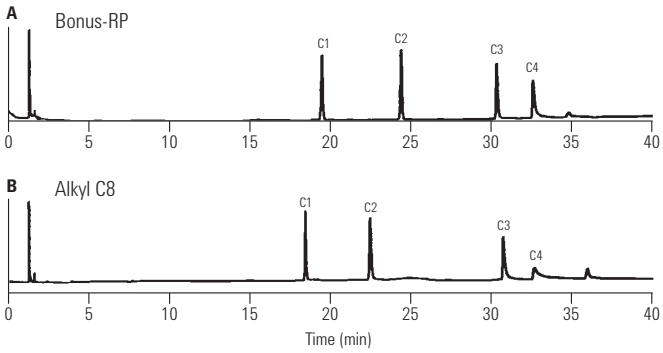
Gradient: 0-100% B in 50 min

Temperature: 40 °C

Detector: 215 nm

Sample: Basic 11-residue peptides with net +1, +2, +3, +4 positive charges at neutral pH

C1: Ac-Gly-Gly-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide
C2: Ac-Lys-Tyr-Gly-Leu-Gly-Gly-Ala-Gly-Gly-Leu-Lys-amide
C3: Ac-Gly-Gly-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide
C4: Ac-Lys-Tyr-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-amide



LCBP020

Peptides: Effect of TFA concentration

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

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

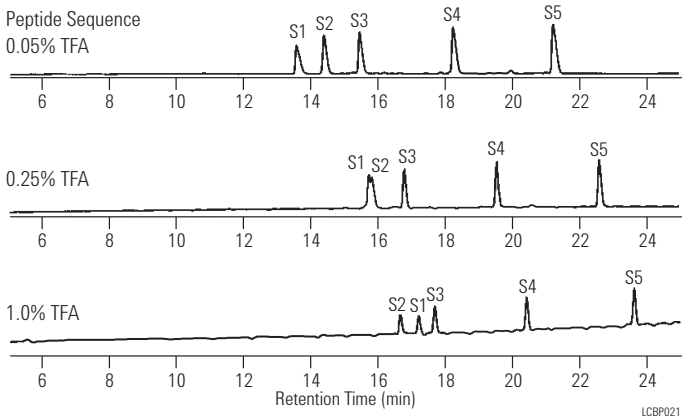
Flow Rate: 1.0 mL/min

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

Temperature: 40 °C

Detector: UV, 254 nm

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



LCBP021

Exploiting chemical stability – TFA concentration

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

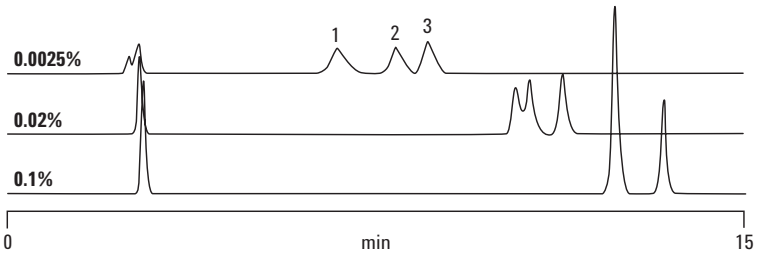
Mobile Phase: A: TFA (various %) in water
B: TFA (various %) in ACN

Gradient: Linear 12-40% B in 15 min

Flow Rate: 1.0 mL/min

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

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



VLC0068

Peptides: Separation of Antiotensins I, II, III with TFA and NH_4OH

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

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

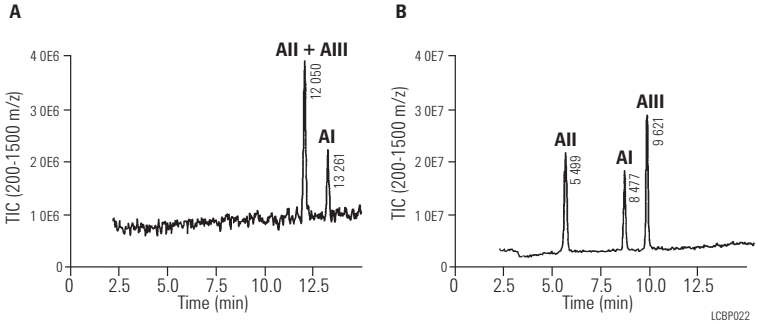
Flow Rate: 0.2 mL/min

Gradient: 15-50% B in 15 min

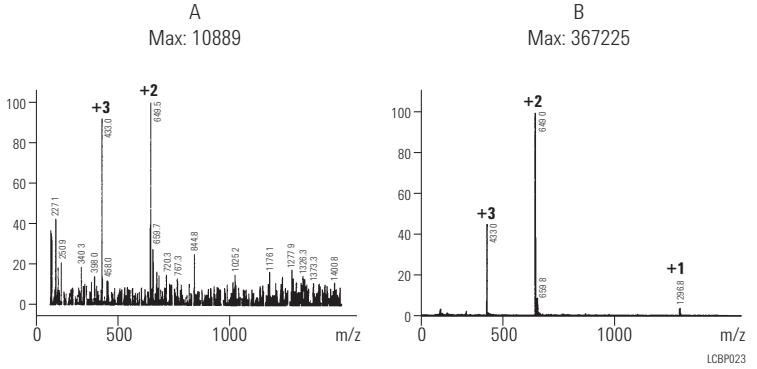
Temperature: 35 °C

MS Conditions: Pos. Ion ESI - Vf 70V, Vcap 4.5 kV
N_2-35 psi, 12 L/min, 325 °C

Sample: 2.5 µL sample (50 pmol each)



LCBP022



LCBP023

Peptides/proteins: Equivalent gradient separations

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

Column: **ZORBAX 300SB-C8**
883750-906
2.1 x 150 mm, 5 µm

Mobile Phase: A: 95% Water: 5% ACN with 0.1% TFA
B: 5% Water: 95% ACN with 0.085% TFA

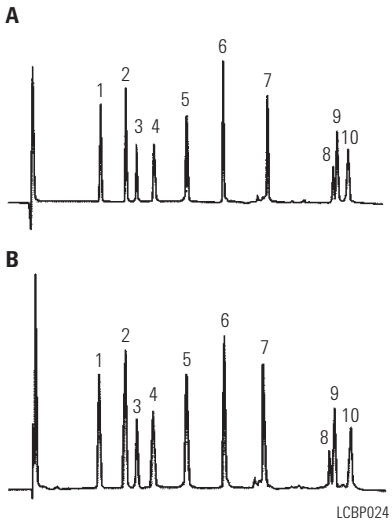
Flow Rate: A: Analytical
1 mL/min
B: Narrow Bore
0.2 mL/min

Gradient: 10-60% B in 30 min

Temperature: 35 °C

Detector: UV, 215 nm

Sample: 10 µL injection, concentration 2-6 µg



LCBP024

1. Met-enkephalin
2. Leu-enkephalin
3. Angiotensin II
4. Neurotensin
5. RNase
6. Insulin (BOV)
7. Lysozyme
8. Calmodulin
9. Myoglobin
10. Carbonic anhydrase

Peptides/proteins: Effect of elevated temperature

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

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

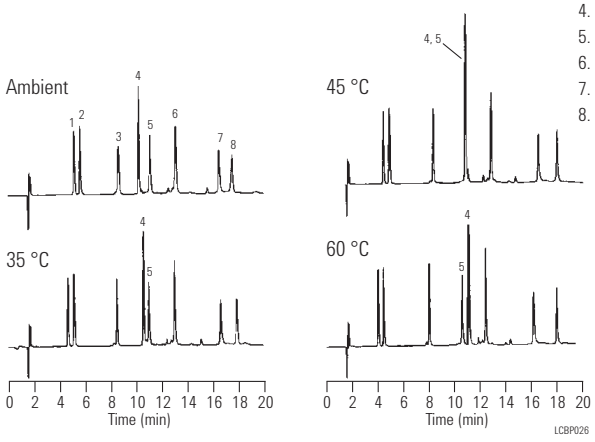
Flow Rate: 1.0 mL/min

Gradient: 15-53% in 20 min, post time 12 min

Temperature: Ambient – 60 °C

Detector: UV, 215 nm

Sample: Polypeptides



LCBP026

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

Separation of polypeptides in under 1 minute

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

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

Flow Rate: 3 mL/min

Gradient: 0-100% B in 1.33 min

Temperature: 70 °C

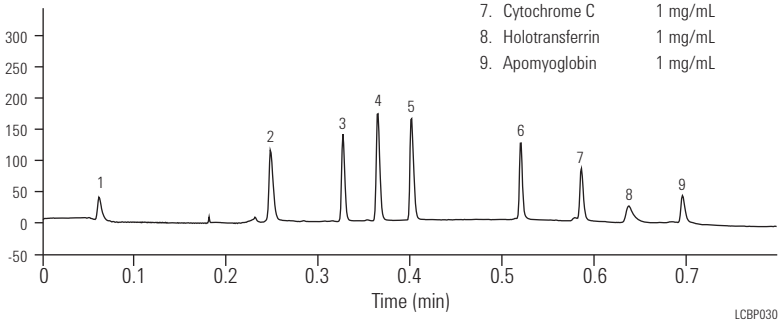
Detector: DAD 215/16 nm, ref = 310/10 nm

Sample: Peptides/proteins, 0.5 µL

Mixer bypassed with P/N G1312-67301; Loop-bypass program

Sample (peptides/proteins)

1.	gly-tyr	0.125 mg/mL
2.	Val-tyr-val	0.5 mg/mL
3.	Met-enkephalin	0.5 mg/mL
4.	Leu-enkephalin	0.5 mg/mL
5.	Angiotensin II	0.5 mg/mL
6.	RNase A	1 mg/mL
7.	Cytochrome C	1 mg/mL
8.	Holotransferrin	1 mg/mL
9.	Apomyoglobin	1 mg/mL



LCBP030

Fast, high-resolution separation of peptides and proteins with Poroshell 300SB-C18

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

Mobile Phase: A: 0.1% TFA
B: 0.07% TFA in ACN

Flow Rate: 3.0 mL/min (360 bar pressure)

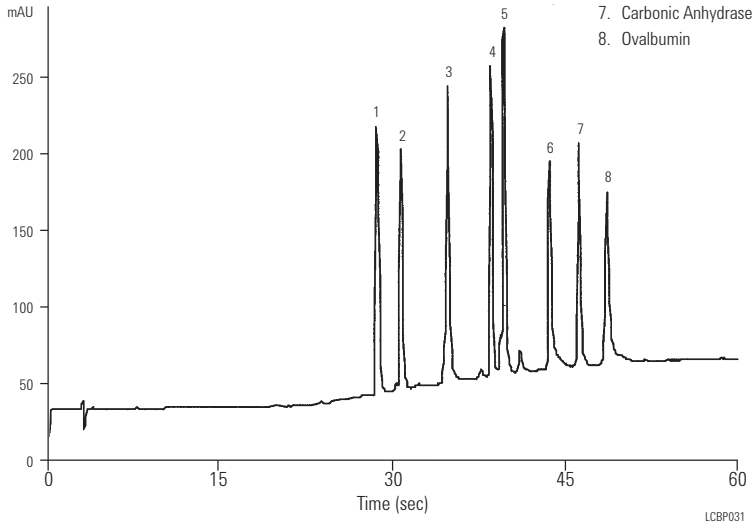
Gradient: 5-100% B in 1.0 min

Temperature: 70 °C

Detector: UV, 215 nm

Spaces between solutes indicate good peak capacity for rapidly separating complex samples.

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



LCBP031

Peptide RP-HPLC/ESI-MS using NH_4OH mobile phase yields both positive and negative ion spectra

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

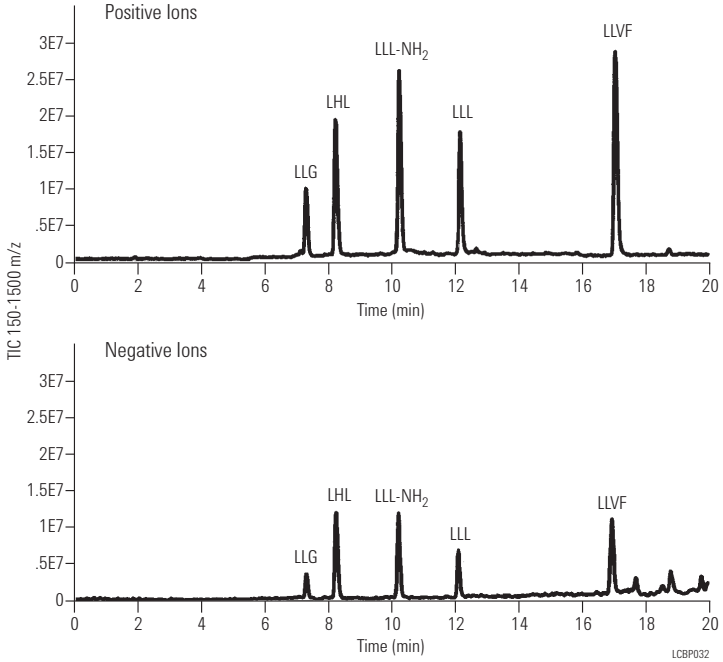
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70 V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min, 300 °C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Comparison of Aβ peptide RP-HPLC separations at low and high pH

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

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

Flow Rate: 0.25 mL/min

Gradient: 29-41% B in 30 min

Temperature: 80 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)

Mobile Phase: A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

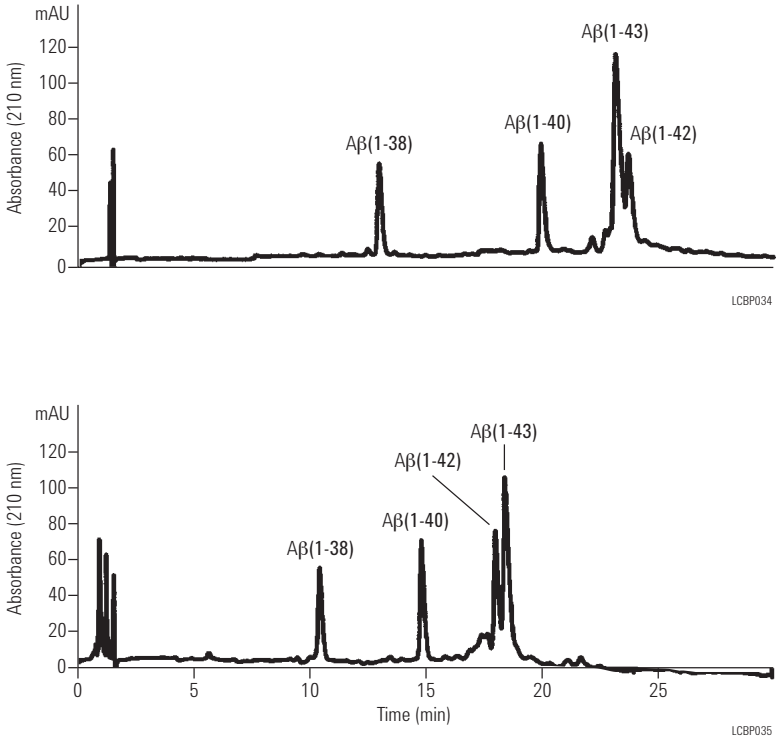
Flow Rate: 0.25 mL/min

Gradient: 26-38% B in 30 min

Temperature: 25 °C

Detector: UV, 210 nm

Sample: 5 µL sample (100 pmol each)



Selectivity comparison of TFA and NH_4OH for peptide RP-HPLC\ESI-MS analysis

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

Mobile Phase: TFA Conditions:
A: 0.1% TFA in water
B: 0.085% TFA in 80% ACN
NH_4OH Conditions:
A: 20 mM NH_4OH in water
B: 20 mM NH_4OH in 80% ACN

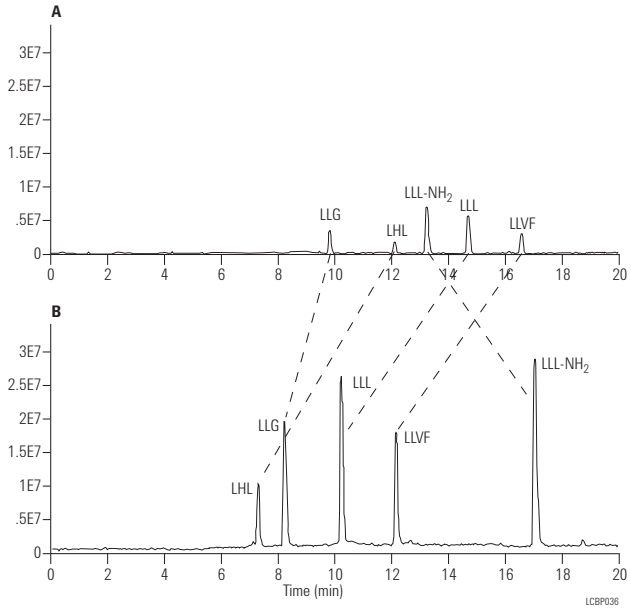
Flow Rate: 0.25 mL/min

Gradient: 5-60% B in 20 min

Temperature: 25 °C

MS Conditions: Pos. Ion ESI – Vf 70V, Vcap 4.5 kV,
N_2 – 35 psi, 12 L/min., 300 °C
TIC 150-1500 m/z

Sample: 4 µL (50 ng each peptide)



Peptide phosphorylation sites LC and LC/MS using Capillary LC columns

Column: **ZORBAX 300SB-C18**
5064-8268
0.5 x 150 mm, 3.5 µm

Mobile Phase: A: Water + 0.1% Formic acid
B: Acetonitrile + 0.1% Formic acid

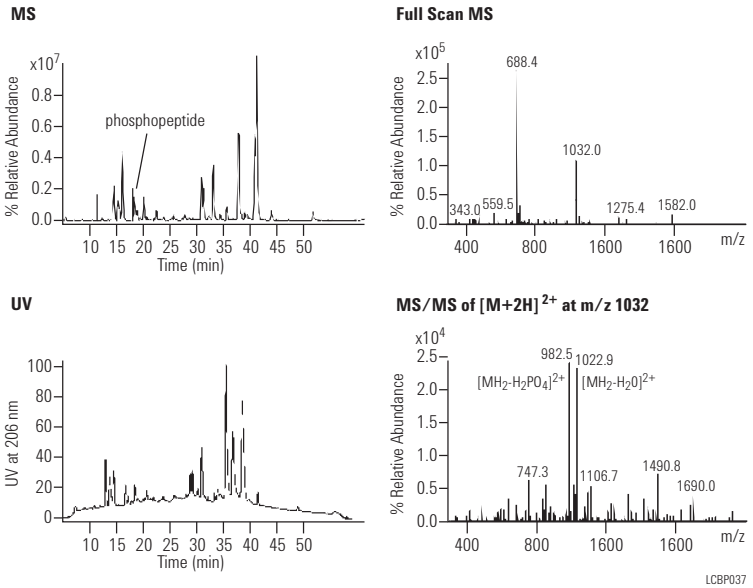
Flow Rate: 5.5 µL/min

Gradient: 5-55% B in 50 min, to
85% B from 55-57 min

Detector: UV, 206 nm

MS Conditions: LC/MS: Pos. Ion ESI with LC/MSD trap
Vcap: 4000 V
Drying gas flow: 7 L/min
Drying gas temperature: 250 °C
Nebulizer: 15 psi
Capillary Exit Volt: 50 V Max
Accum Time: 300 ms
Total Averages: 3
Isolation Width: 3 m/z
Frag Ampitude: 1.0 V

Sample: Beta case in digest, 100 nL (4 pmol)



Proteins: Effect of bonded phase, RP

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

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

Mobile Phase: A: 0.1% TFA in Water,
B: 0.1% TFA in 50/50 ACN/Water

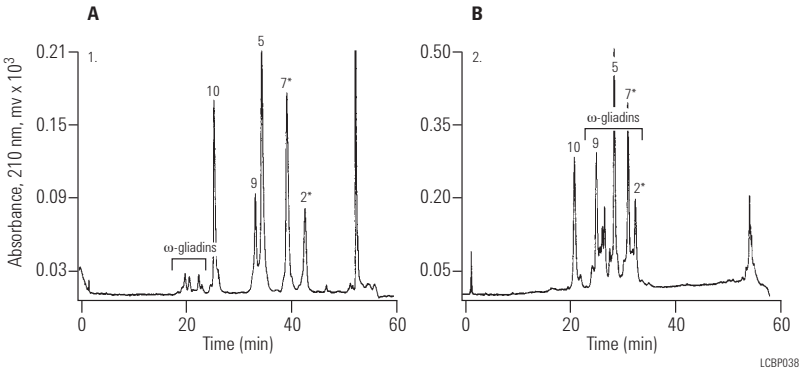
Flow Rate: 1.0 mL/min

Gradient: 1. 46-96% B in 60 min 23-48% ACN
2. 50-86% B in 60 min 25-43% ACN

Temperature: 50 °C

Detector: UV, 210 nm

Sample: Wheat proteins, including w-gliadins



Proteins: Effect of bonded phase

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

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

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

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

Mobile Phase: A: 0.1% TFA in H_2O
B: 0.09% TFA in 80% ACN/20% Water

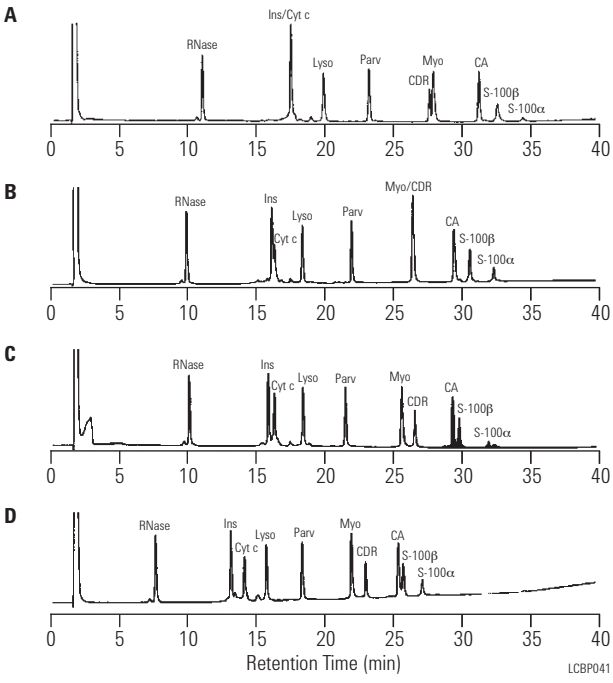
Flow Rate: 1.0 mL/min

Gradient: 25-70% B in 40 min

Temperature: 60 °C

Detector: UV, 210 nm

Sample: Polypeptides, 3 µg each



Standard proteins by reversed-phase

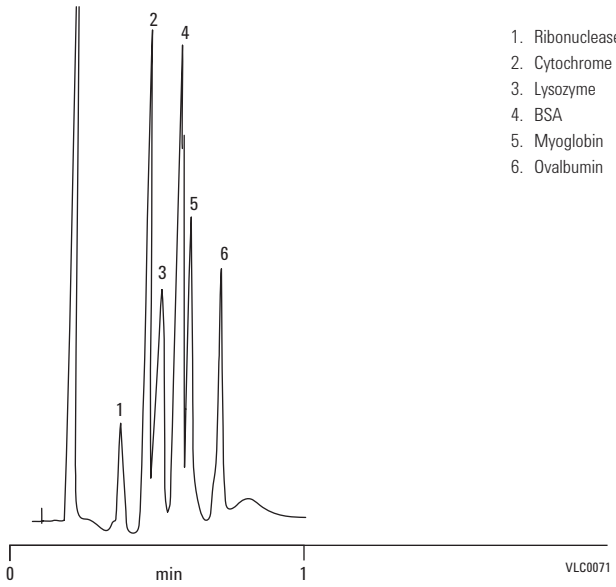
Column: **PLRP-S 4000Å**
PL1512-1803
4.6 x 50 mm, 8 µm

Mobile Phase: A: 0.1% TFA in 95% water:5% ACN
B: 0.1% TFA in 5% water:95% ACN

Gradient: Linear 18-60% B in 1 min

Flow Rate: 4.0 mL/min

Detector: UV, 280 nm



1. Ribonuclease A
2. Cytochrome C
3. Lysozyme
4. BSA
5. Myoglobin
6. Ovalbumin

Standard ion-exchange protein separation

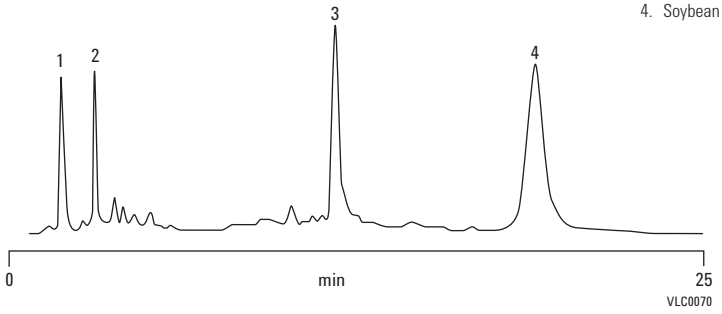
Column: **PL-SAX 1000Å**
PL1551-1502
4.6 x 50 mm, 5 µm

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

Gradient: 0-100% B in 20 min

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm



1. Myoglobin
2. Bovine carbonic anhydrase
3. Ovalbumin
4. Soybean trypsin inhibitor

Deoxynucleosides: Using rapid resolution 3.5 µm columns

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

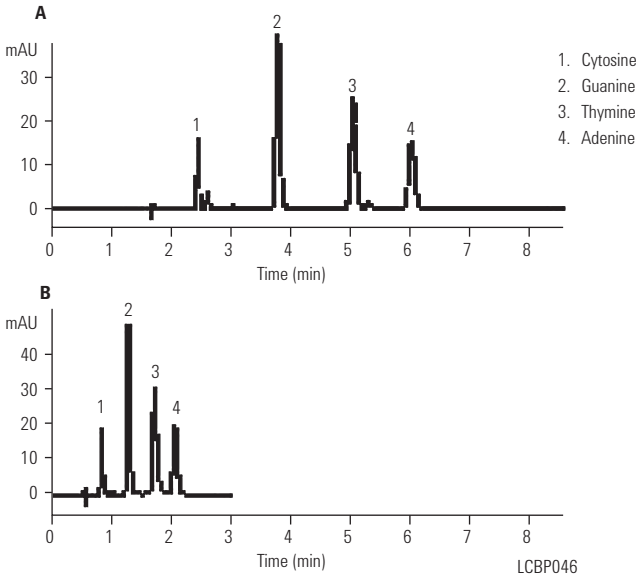
Column B: **ZORBAX SB-CN**
835975-905
4.6 x 50 mm, 3.5 µm

Mobile Phase: A: 0.1% TFA
B: 90/10 v/v Methanol/Water (0.1% TFA)
Isocratic, 97.5% A, 2.5% B

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 254 nm



BSA tryptic digest on RRHT

Column: **ZORBAX SB-C18**
820700-902
2.1 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

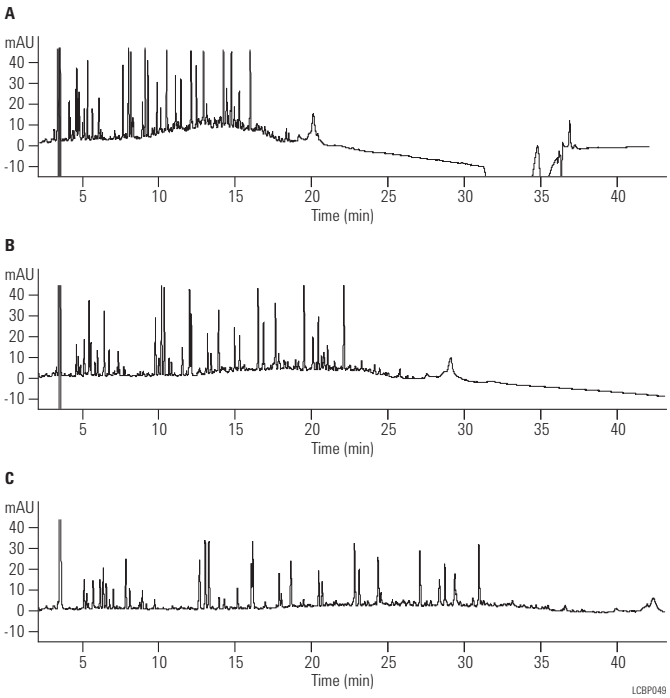
Flow Rate: 0.5 mL/min

Gradient: A: Time 0% B 5 min, Time 30% B 60 min
B: Time 0% B 5 min, Time 45% B 60 min
C: Time 0% B 5 min, Time 67.5% B 60 min

Temperature: 80 °C

Detector: UV, 214 nm

Sample: BSA tryptic digest



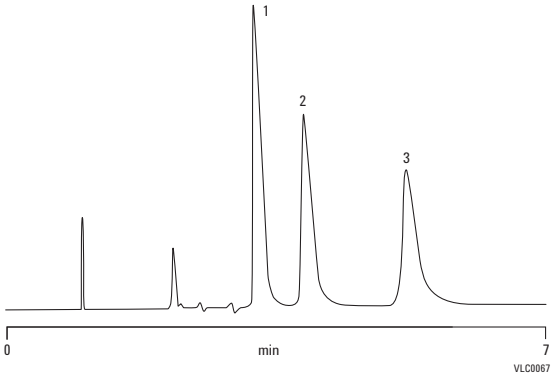
Catecholamines

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

Mobile Phase: 95% 25 mM citric acid, 25 mM Na_2HPO_4, 1 mM heptane sulfonic acid:5% ACN, pH 2.85

Flow Rate: 1.0 mL/min

Detector: UV, 280 nm



1. Noradrenaline
2. Adrenaline
3. Dopamine

Whey proteins in dairy samples – milk

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

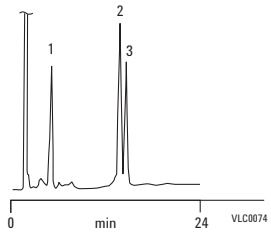
Mobile Phase: A: 0.1% TFA in 99% water:1% ACN
B: 0.1% TFA in 1% water:99% ACN

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

Flow Rate: 1.0 mL/min

Injection Volume: 10 µL

Detector: UV, 220 nm



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

Temperature as a tool to enhance mass transfer and improve resolution of oligonucleotides in ion-pair reversed-phase HPLC

Column: **PLRP-S 100Å**
PL1512-1300
4.6 x 50 mm, 3 µm

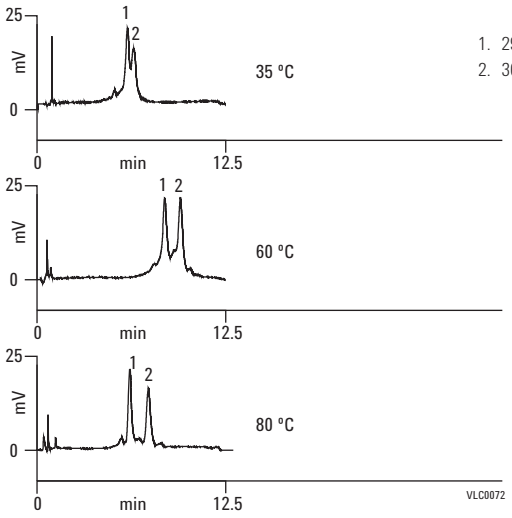
Mobile Phase: A: 100 mM TEAA
B: 100 mM TEAA in 25% ACN

Gradient: 5% change in buffer B over 5 min

Flow Rate: 1.0 mL/min

Temperature: 35 °C, 60 °C, or 80 °C

Detector: UV, 254 nm



1. 29-mer
2. 30-mer

Hydrophilic purine/pyrimidine separation

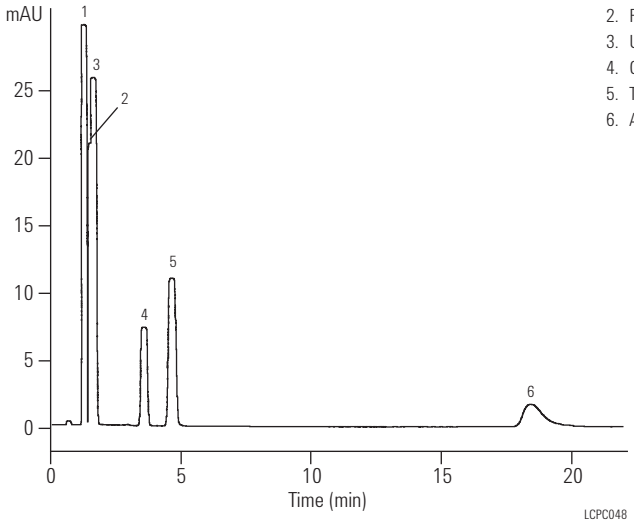
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 50 mM NaOAc, pH 4.6

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



1. Cytosine
2. Fluorocytosine
3. Uracil
4. Guanine
5. Thymine
6. Adenine

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Chemical/Industrial Applications

Analysis of biocides in hand sanitizer

Column: **ZORBAX RRHD Eclipse Plus C18**
959757-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: H_2O (0.5% TFA)
B: ACN (0.04% TFA)

Flow Rate: 1.7 mL/min

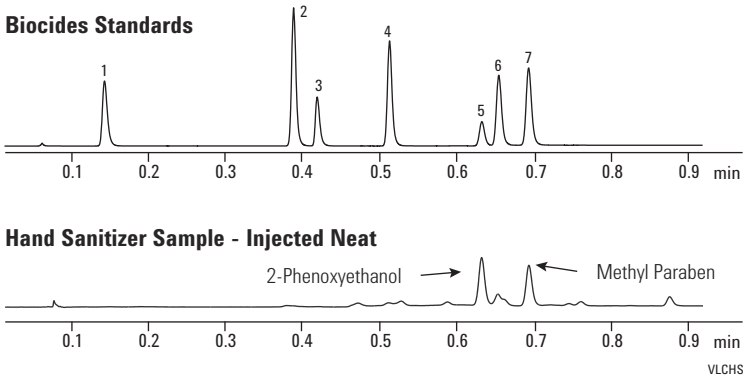
Gradient:

Time 0.0	95/5 A/B	
Time 1.0	55/45 A/B	
Time 1.1	0/100 A/B	

DAD: 275 nm (0 min)
225 nm (0.46 min)
255 nm (0.67 min)

Sample: 1 µL injection of 50 ppm std.

Temperature: 30 °C



1. Kathon 1A
2. Kathon 1B
3. Carbendazim
4. 1,2-Benzisothiazol-3(2H)-one
5. 2-Phenoxyethanol
6. Benzoic Acid
7. Methyl Paraben

Triton X-114: Decreasing run-time by changing bonded phase

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

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

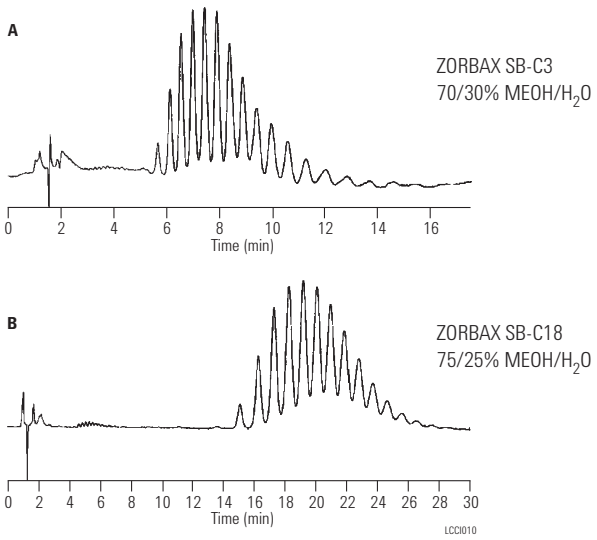
Mobile Phase: MeOH and H_2O (as indicated)

Flow Rate: 1.0 mL/min

Temperature: 50 °C

Detector: UV, 225 nm

Sample: Triton X-114



Organic acids separated on ZORBAX SB-Aq

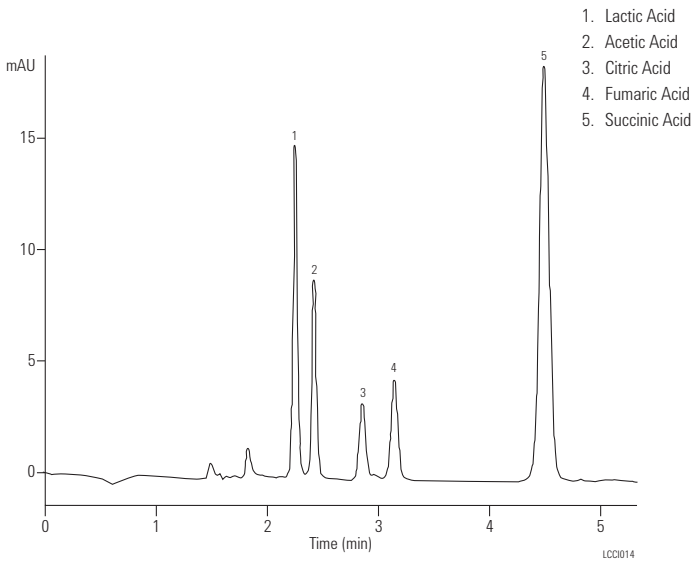
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 99% 20 mM NaH_2PO_4, pH 2, 1% ACN

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 210 nm



Brij 35

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

Mobile Phase: A: Water
B: ACN

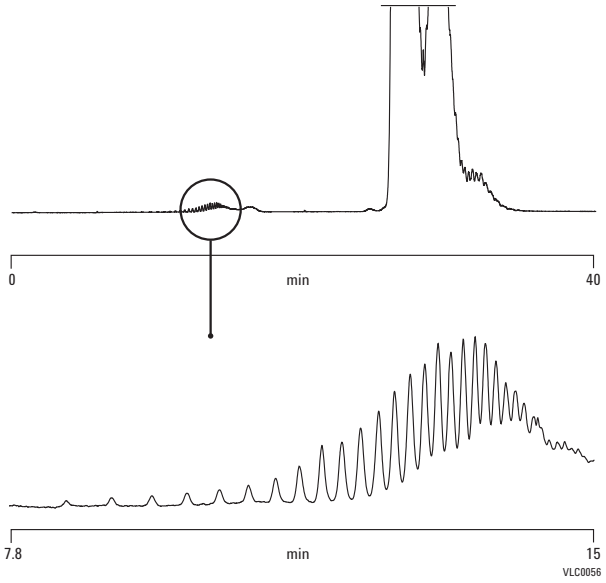
Gradient: 0-100% B in 40 min

Flow Rate: 0.8 mL/min

Injection Volume: 10 µL

Sample Conc: 1 mg/mL

Detector: ELS (neb=50 °C, evap=70 °C, gas=1.5 SLM)



Alcohols and aliphatic compounds

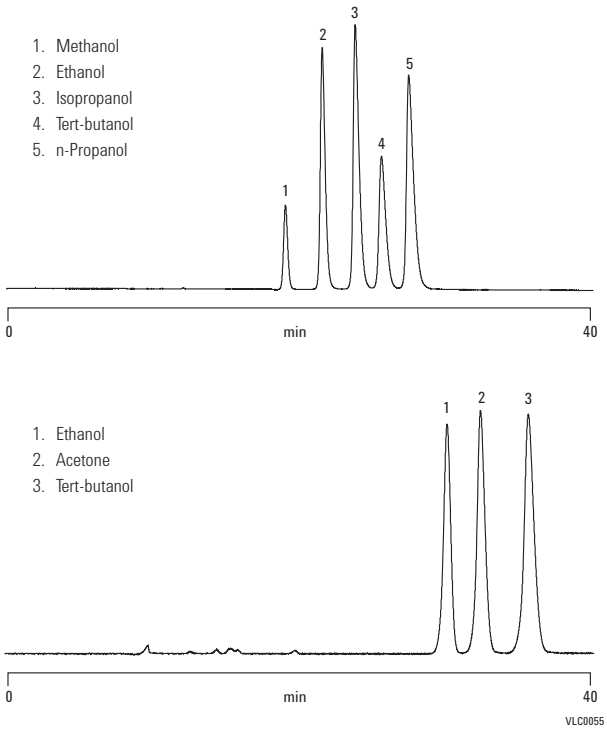
Column: **Hi-Plex H**
PL1170-6830
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 40 °C

Detector: 356-LC RI



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Environmental Applications

NEW!

Fast LC/MS/MS analysis of group 4 pharmaceuticals from EPA-1694

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

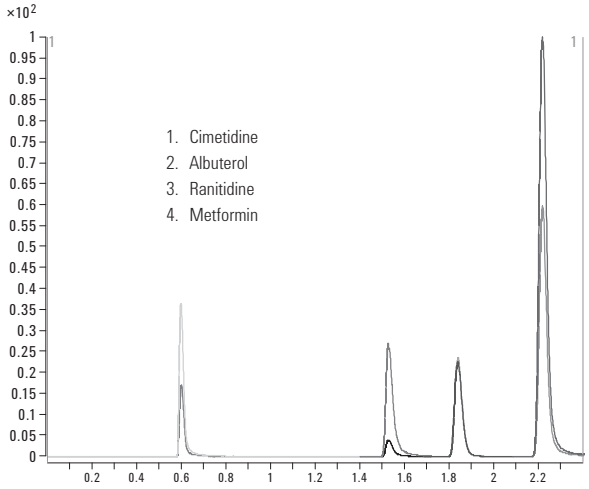
Mobile Phase: A: 10 mM ammonium acetate in water, pH 6.7
B: acetonitrile

Flow Rate: 1 mL/min

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

MS Conditions: TCC: 25 °C
dMRM, ESI positive mode, cycle time 35 ms
Drying Gas: 9 L/min, 300 °C
Nebulizer Pressure: 40 psig
Capillary Voltage: 4000

Sample: 0.1 µL injection of 0.1 mg/mL each in acetonitrile/water (3:1): cimetidine, albuterol, ranitidine and metformin



NEW!

Separation of azo dye degradation products

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

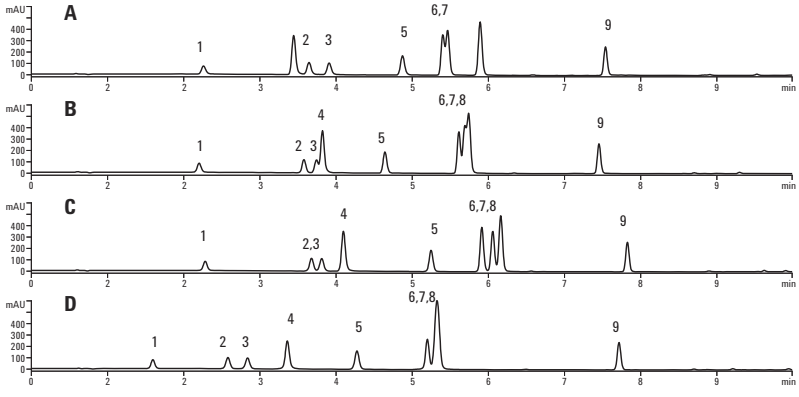
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Flow Rate: 0.4 mL/min

Gradient: 15 to 100% MeOH over 10 min

Solvent: 10 mM Ammonium acetate, pH 4.8

1. Aniline
2. o-Toluidine
3. Methoxyaniline
4. Chloroaniline
5. Benzidine
6. Dimethylbenzidine
7. 3,3'-Dimethoxybenzidine
8. Napthylamine
9. Dichlorobenzidine



Comparison of phenols separation with Poroshell 120

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

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
0.8	5%
6.8	60%

1200 SL controlled temperature
at 25 °C 2 mm flow cell

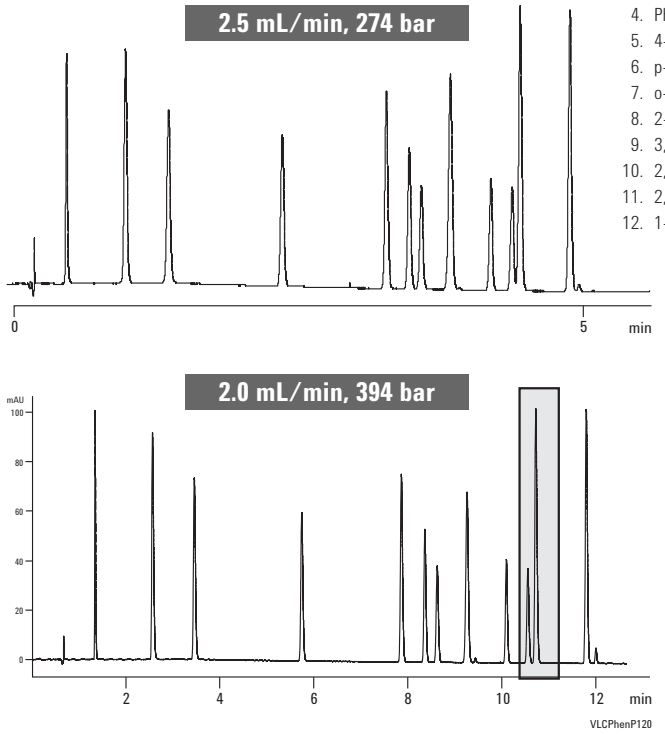
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: A: Water with 0.1% Formic Acid
B: Acetonitrile

Gradient:

Time	%B
2.0	5%
17	60%

1200 RRLC SL controlled temperature
at 25 °C 2 mm flow cell



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

DNPH: Derivatized Aldehydes obtained from air

Column: **ZORBAX ODS**
884950-543
4.6 x 250 mm, 5 µm

Mobile Phase: A: 100% Water
B: 100% ACN

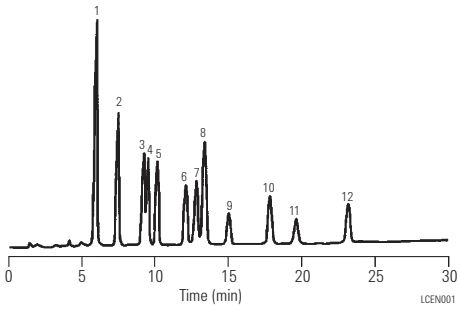
Flow Rate: 1.0 mL/min

Gradient: 60-75% B in 30 min; Wash: From 75-100% B in 5 min, after 5 min return to 60% B

Temperature: 35 °C

Detector: UV, 230 nm

Sample: DNPH Derivatized Aldehydes



1. Formaldehyde – DNPH
2. Acetaldehyde – DNPH
3. Acetone – DNPH
4. Acrolein – DNPH
5. Propionaldehyde – DNPH
6. Crotonaldehyde – DNPH
7. 2-Butanone (MEK) – DNPH
8. Methacrolein – DNPH
   n-Butyraldehyde – DNPH
9. Benzaldehyde – DNPH
10. Valeraldehyde – DNPH
11. m-Tolualdehyde – DNPH
12. Hexaldehyde – DNPH

Amitrol in water by LC/MS, 0.05 ppb

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

Mobile Phase: A: 10 mM ammonium acetate
B: MeOH

Flow Rate: 0.4 mL/min

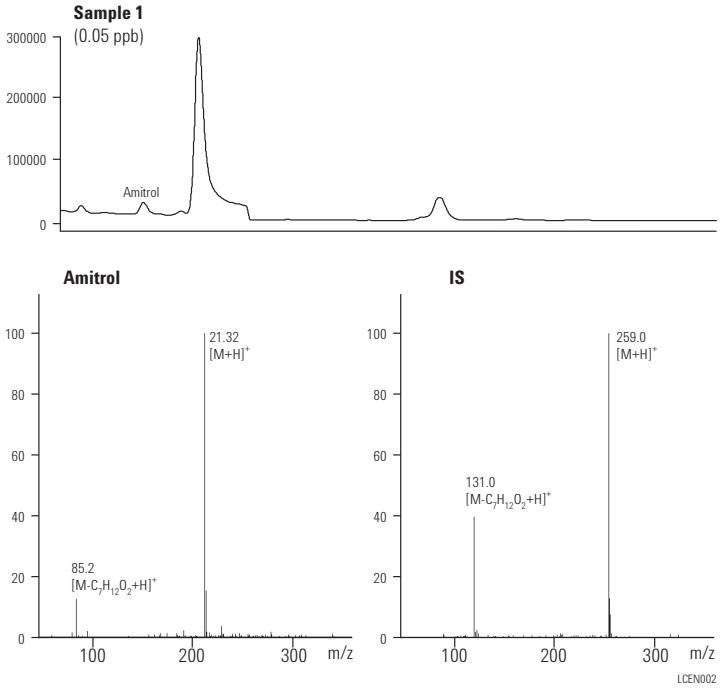
Gradient: 0 min, 65% B; 10 min, 65% B;
15 min, 100% B; 20 min, 65% B

Temperature: 30 °C

MS Conditions:

Ionization Mode:	APCI, positive polarity
SIM parameters:	Ion: 213 Amitrol
Ion:	259 IS
Fragmentor:	100 V
SIM Resolution:	Low
Vaporizer:	325 °C
Drying Gas (N_2):	5.0 L/min
Gas Temperature:	350 °C
Nebulizer pressure:	60 psig
Vcap:	4000 V
Corona:	4.0 uA

Sample: Amitrol in water, 100 µL



Anilines, substituted: Rapid separation

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

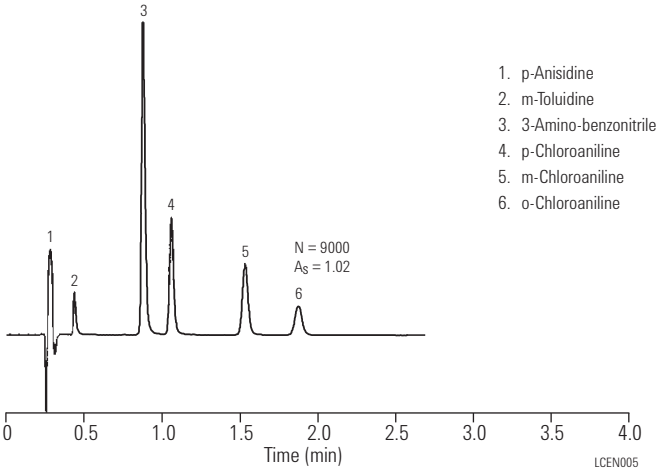
Mobile Phase: 20% ACN/80% 25 mM phosphate buffer, pH 2.5

Flow Rate: 3.0 mL/min

Temperature: 60 °C

Detector: UV, 254 nm

Sample: Anilines



1. p-Anisidine
2. m-Toluidine
3. 3-Amino-benzonitrile
4. p-Chloroaniline
5. m-Chloroaniline
6. o-Chloroaniline

Explosives and related compounds: Qualitative and quantitative analysis

Column A: **ZORBAX SB-C18**
883700-922
2.1 x 150 mm, 5 µm

Column B: **ZORBAX SB-CN**
883700-905
2.1 x 150 mm, 5 µm

Mobile Phase: A = ACN + 5% H_2O + 5 mM CF_3COONH_4
B = H_2O + 5% ACN + 5 mM CF_3COONH_4,
pH 2.7 (CF_3COOH)

Flow Rate: 0.23 mL/min

Gradient: A:
0 min 80% B
2 min 80% B
10 min 70% B
20 min 65% B
25 min 60% B
35 min 30% B
40 min 30% B
42 min 80% B

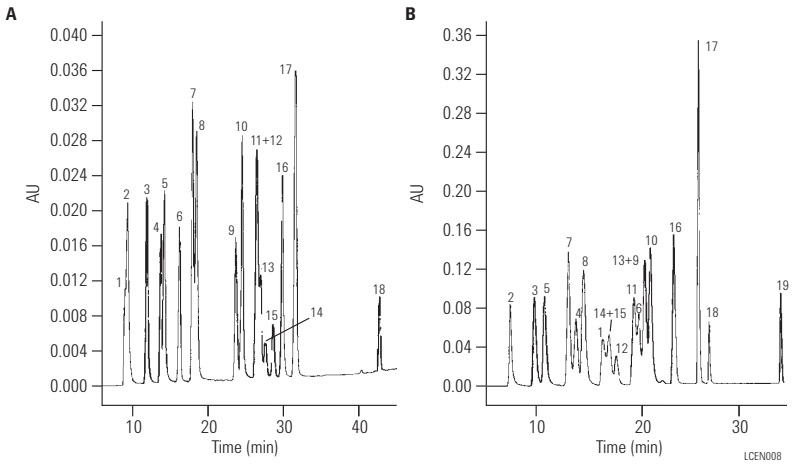
B:
0 min 80% B
1 min 80% B
15 min 70% B
30 min 20% B
35 min 20% B
37 min 80% B

Temperature: 18 °C

Detector: UV, 210, 240, 360 nm, wavelength switching for each compound

Sample: 10 µL of 19 explosive compounds in ACN/H_2O (20/80)

1. Picric acid
2. 4-Amino-2-nitrotoluene
3. 2-Amino-6-nitrotoluene
4. RDX
5. 2-Amino-4-nitrotoluene
6. HMX
7. 1,3-Dinitrobenzene
8. 1,3,5-Trinitrobenzene
9. 2-Amino-4,6-dinitrotoluene
10. 2,4-Dinitrotoluene
11. 4-Amino-4,6-dinitrotoluene
12. 2-Nitrotoluene
13. 2,6-Dinitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene
16. 2,4,6-Trinitrotoluene
17. Tetryl
18. Diphenylamine
19. Hexyl



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Explosives from soil extract

Column: **ZORBAX SB-C18**
880975-302
3.0 x 250 mm, 5 µm

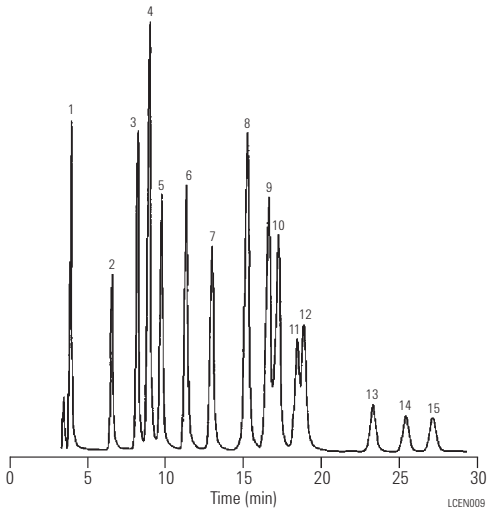
Mobile Phase: Methanol/Water (50/50) (v/v)

Flow Rate: 0.3 mL/min

Temperature: Ambient

Detector: UV, 230 nm

Sample: 10 µL explosives mix



1. Octogen (HMX)
2. Hexogen (RDX)
3. 2-Amino-6-nitrotoluene
4. 1,3,5-Trinitrobenzene
5. 2-Amino-4-nitrotoluene
6. 1,3-Dinitrobenzene
7. Tetryl
8. 2,4,6-Trinitrotoluene
9. 4-Amino-2,6-dinitrotoluene
10. 2-Amino-4,6-dinitrotoluene
11. 2,6-Dinitrotoluene
12. 2,4-Dinitrotoluene
13. 2-Nitrotoluene
14. 4-Nitrotoluene
15. 3-Nitrotoluene

Herbicides on different bonded phases

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

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

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

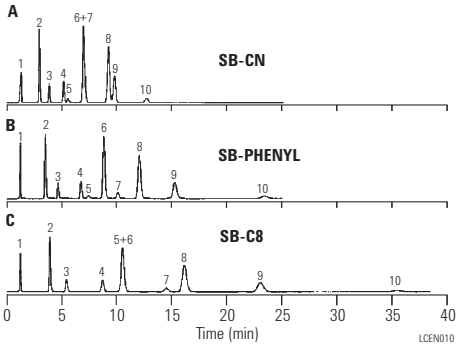
Mobile Phase: 35% ACN, 65% Water

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

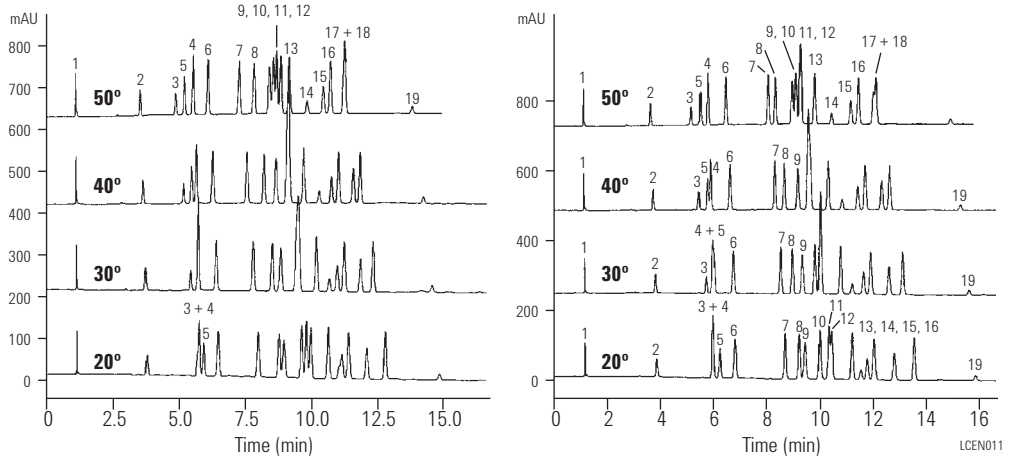
Sample: Herbicides



1. Bentazon
2. Tebuthiuron
3. Simazine
4. Atrazine
5. Prometon
6. Diuron
7. Propazine
8. Propanil
9. Prometryne
10. Metolachlor

Herbicide/pesticide standards: Effect of bonded phase

	Column: Eclipse XDB-C8 993967-906 4.6 x 150 mm, 5 µm	Column: Eclipse XDB-C18 993967-902 4.6 x 150 mm, 5 µm
Mobile Phase:	Water/Acetonitrile	Water/Acetonitrile
Flow Rate:	1.0 mL/min	1.0 mL/min
Gradient:	20-60% in 15 min	20-60% in 15 min
Temperature:	50 °C 40 °C 30 °C 20 °C	50 °C 40 °C 30 °C 20 °C
Detector:	DAD 240	DAD 240
Sample:	Herbicide & pesticide standards	Herbicide & pesticide standards



1. Desethyldesisopropylatrazine
2. Desethylatrazine
3. Benzthiazuron
4. Hexazinon
5. Metoxuron
6. Simazine
7. Methabenzthiazuron
8. Simazine
9. Atrazine
10. Isoproturon
11. Diuron
12. Monolonuron
13. Metobromuron
14. Metazachlor
15. Propazine
16. Sebutylazine
17. Terbutylazine
18. Linuron
19. Metolachlor

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Separation of EPA 610 PAH Mix

Column: **Eclipse PAH**
959990-318
3.0 x 250 mm, 5 µm

Mobile Phase: A: Water
B: Acetonitrile
Initial %B = 40

Flow Rate: 0.85 mL/min

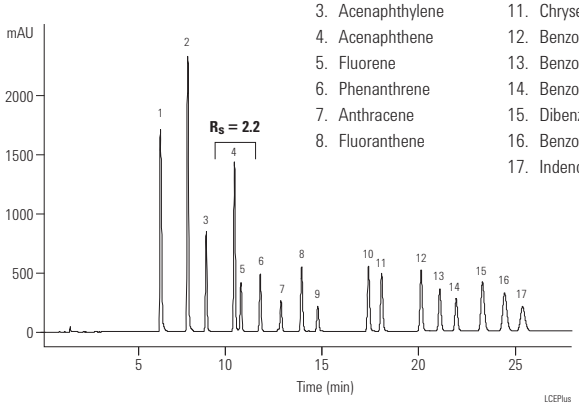
Gradient:

Time (Min)	%B
0.00	45
17.5	100
24.0	100
25.5	40
27.5	40

Stop Time = 25.0

Temperature: 25 °C

Detector: 220, 4 nm No Ref.; Stop time = 26.0 min



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benzo(a)anthracene
11. Chrysene
12. Benzo(b)fluoranthene
13. Benzo(k)fluoeanthene
14. Benzo(a)pyrene
15. Dibenzo(a,h)anthracene
16. Benzo(g,h,i)perylene
17. Indeno(1,2,3-c,d)pyrene

Polycyclic aromatic hydrocarbons according to EPA Method 610

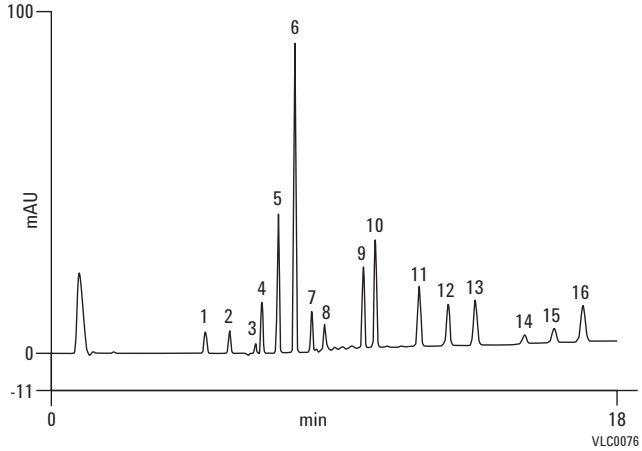
Column: **Pursuit PAH**
A7001100X046
4.6 x 100 mm, 3 µm

Sample: NIST 16473 Standard

Mobile Phase: A: ACN:water, 25:75
B: ACN

Flow Rate: 2.0 mL/min

Detector: UV, 254 nm



1. Naphthalene
2. Acenaphthylene
3. Aceneaphthene
4. Fluorene
5. Phenanthrene
6. Anthracene
7. Fluoranthene
8. Pyrene
9. Benzo[a]anthracene
10. Chrysene
11. Benzo[b]fluoranthene
12. Benzo[k]fluoranthene
13. Benzo[a]pyrene
14. Dibenzo[a,h]anthracene
15. Benzo[ghi]perylene
16. Indeno[1,2,3-cd]pyrene

NEW!

Rapid method development for 18 PAH compounds with an Agilent RRHD Eclipse PAH column

Column: **ZORBAX RRHD Eclipse PAH**
959758-918
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: Water
B: Acetonitrile

Flow Rate: 0.84 mL/min

Gradient: 40-100% B, gradient time (t_g) varies from 1 to 20 min; isocratic hold at 100% B for 2 min, re-equilibrate column at 40% B for 3 min

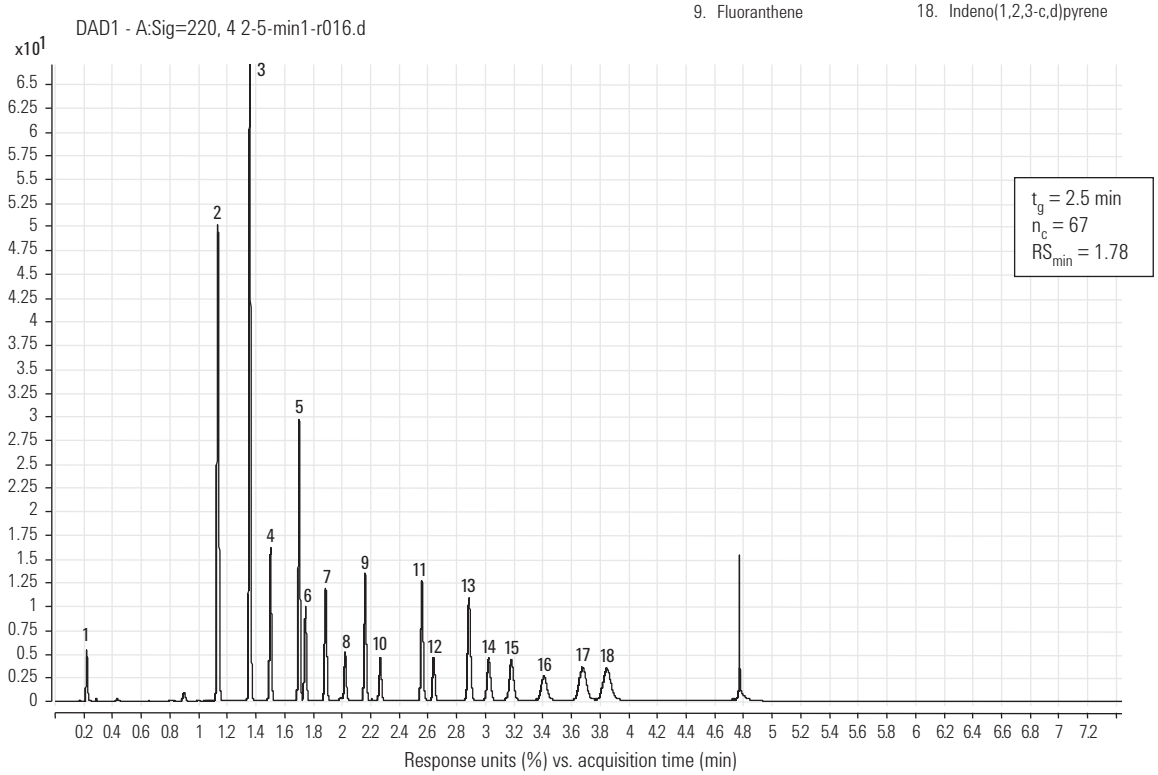
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC

MS Conditions: Sig = 220, 4 nm; Ref = Off

Sample: 0.5 µL injection of diluted Agilent PAH Mixture (P/N 8500-6035) spiked with thiourea as a v_0 marker

1. Thiourea (V_0 marker)
2. Toluene
3. Naphthalene
4. Acenaphthylene
5. Acenaphthene
6. Fluorene
7. Phenanthrene
8. Anthracene
9. Fluoranthene
10. Pyrene
11. Benzo(a)anthracene
12. Chrysene
13. Benzo(b)fluoranthene
14. Benzo(k)fluoranthene
15. Benzo(a)pyrene
16. Dibenzo(a,h)anthracene
17. Benzo(g,h,i)perylene
18. Indeno(1,2,3-c,d)pyrene



Gradient times are rapidly screened for the separation of 18 compounds.

Separation of 20 PAHs on Eclipse PAH

Column: **Eclipse PAH**
959964-918
4.6 x 100 mm, 1.8 µm

Mobile Phase: A: Water
B: Acetonitrile

Flow Rate: 1.8 mL/min

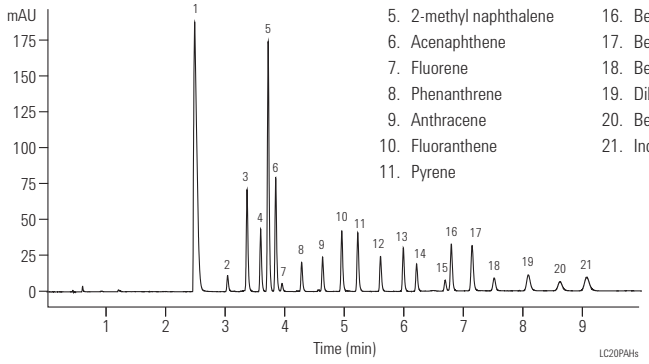
Gradient:

Time (Min)	% B
0	40
6	100
9.5	100
10	40

Stop Time = 12

Temperature: 25 °C

Detector: 230, 8 nm No Ref.; Data rate 0.2 s, micro flow cell



1. Toluene
2. Naphthalene
3. Acenaphthylene
4. 1-methyl naphthalene
5. 2-methyl naphthalene
6. Acenaphthene
7. Fluorene
8. Phenanthrene
9. Anthracene
10. Fluoranthene
11. Pyrene
12. Terphenyl-d14
13. Benzo(a)anthracene
14. Chrysene
15. Benz(e)pyrene
16. Benzo(b)fluoranthene
17. Benzo(k)fluoranthene
18. Benzo(a)pyrene
19. Dibenzo(a,h)anthracene
20. Benzo(g,h,i)perylene
21. Indeno(1,2,3-c,d)pyrene

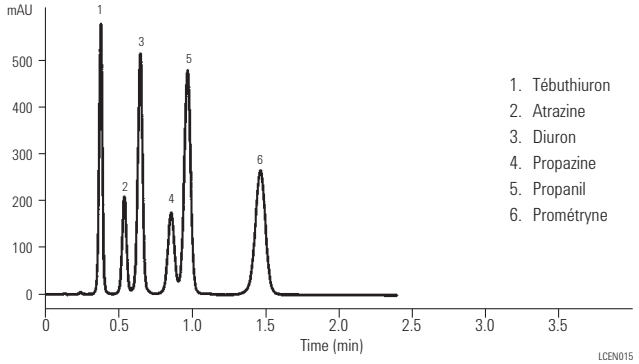
Herbicides: Rapid separation

Column: **Eclipse XDB-C18**
933975-902
4.6 x 30 mm, 3.5 µm

Mobile Phase: MeOH:H_2O (60:40)

Flow Rate: 2 mL/min

Temperature: Ambient



1. Tébuthiuron
2. Atrazine
3. Diuron
4. Propazine
5. Propanil
6. Prométryne

Phenoxyacid herbicides

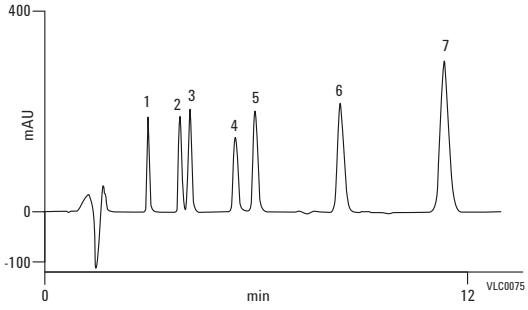
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: MeCN:water+0.1% HCOOH, 50:50

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm



1. Phenoxyacetic acid
2. o-Chlorophenoxyacetic acid
3. p-Chlorophenoxyacetic acid
4. 2,3-Dichlorophenoxyacetic acid
5. 2,4-Dichlorophenoxyacetic acid.
6. 2,4,5-Trichlorophenoxyacetic acid
7. 2,4,5-Trichlorophenoxypropionic acid (Silvex)

Triazine pesticides on Bonus-RP and Alkyl C8 phase

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

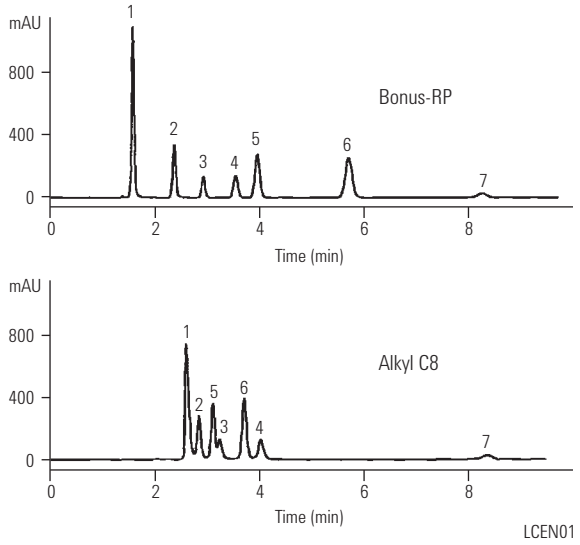
Mobile Phase: MeOH: 0.1% TFA (70:30)*

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: 254 nm

Sample: Triazine pesticides, 2 µL
1. Prometryne
2. Tebuthiuron
3. Atrazine
4. Propazine
5. Diuron
6. Propanil
7. Dacthal



LCEN01

* For low pH work with Bonus-RP, a TFA mobile phase is often preferred over phosphate, and is compatible with LC/MS.

Phenols, substituted

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

Mobile Phase: 20% ACN/80% 0.01 M H_3PO_4 to 45% ACN in 7.5 min

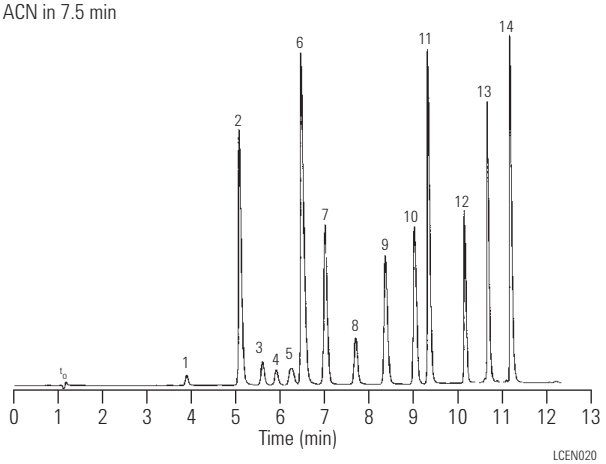
Flow Rate: 1.5 mL/min

Gradient: 80% ACN in 2.0 min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Phenols



LCEN020

1. Phenol
2. 4-Nitrophenol
3. m-Cresol
4. o-Cresol
5. 2-Chlorophenol
6. 2,4-Dinitrophenol
7. 2-Nitrophenol
8. 2,4-Dimethylphenol
9. 4-Chloro-3-methylphenol
10. 2,4-Dichlorophenol
11. 2-Methyl-4,6-dinitrophenol
12. 2,4,6-Trichlorophenol
13. 2,3,4,6-Tetrachlorophenol
14. Pentachlorophenol

Plant hormones: Rapid gradient elution separation

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

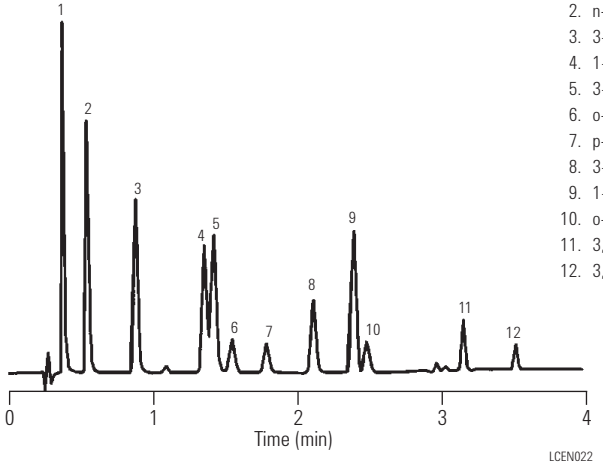
Mobile Phase: A: Water with 0.1% TFA
B: Acetonitrile with 0.1% TFA

Flow Rate: 3.0 mL/min

Temperature: 60 °C

Detector: UV, 245 nm

Sample: Plant hormones



1. Kinetin
2. n-6-Benzyl adenine
3. 3-Indole acetic acid
4. 1-Naphthyl acetamide
5. 3-Indole proprionic acid
6. o-Chlorophenoxy acetic acid
7. p-Chlorophenoxy acetic acid
8. 3-Indole butyric acid
9. 1-Naphthyl acetic acid
10. o-Chlorophenoxy proprionic acid
11. 3,4,5-Trichlorophenoxy acetic acid
12. 3,4,5-Trichlorophenoxy proprionic acid

VX nerve agent metabolites by LC/MS-IS standard (C13 labeled)

Column: **ZORBAX NH2**
860700-708
2.1 x 50 mm, 5 µm

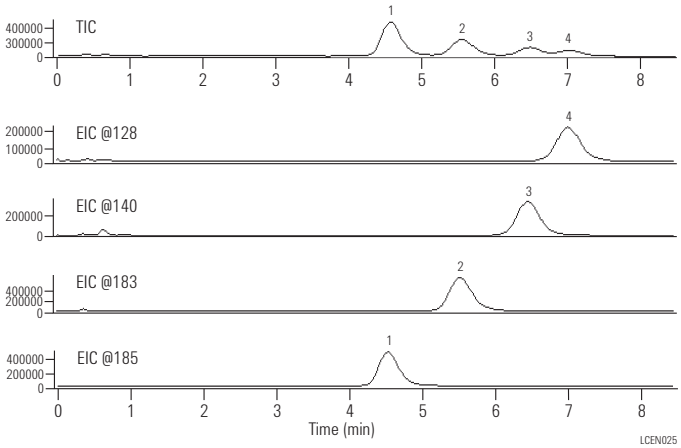
Mobile Phase: 1:1 (20 mM Ammonium Actetate pH 4.5/Acetonitrile)

Flow Rate: 0.5 mL/min, 1 µL injection (prepared std in ACN)

Temperature: 35 °C

Detector: ESI-Negative Ion, Gas Flow 12 L/min, Nebulizer 60 psi

Sample	MW
1. Cyclohexyl methylphosphonic acid	178
2. Pinacolyl methylphosphonic acid	180
3. Isopropyl methylphosphonic acid	138
4. Ethyl methylphosphonic acid	124



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Food and Consumer Product Applications

NEW!

Blueberry anthocyanin analysis

Column A: **Poroshell 120 SB-C18**
687975-902
4.6 x 75 mm, 2.7 µm

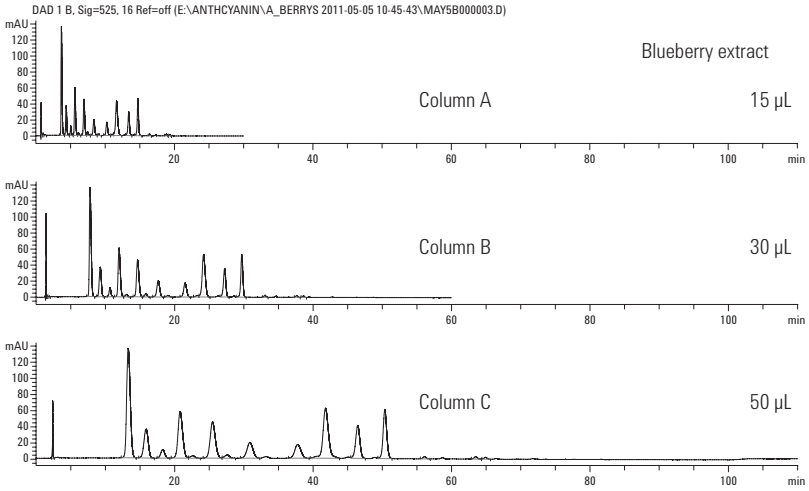
Column B: **ZORBAX SB-C18**
863953-902
4.6 x 150 mm, 3.5 µm

Column C: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

Flow Rate: 1 mL/min

Detector: Agilent 1260 Rapid Infinity LC

Blueberry anthocyanin analysis on totally porous and superficially porous StableBond C18 columns. Overlay of anthocyanin method with 250 mm 5 µm, 150 mm 3.5 µm, and 75 mm 2.7 µm at 1 mL/min.



NEW!

Analysis of pesticide residues in green tea

Column: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Mobile Phase: A: 5 mM FA in water
B: 5 mM FA in ACN

Flow Rate: 0.4 mL/min

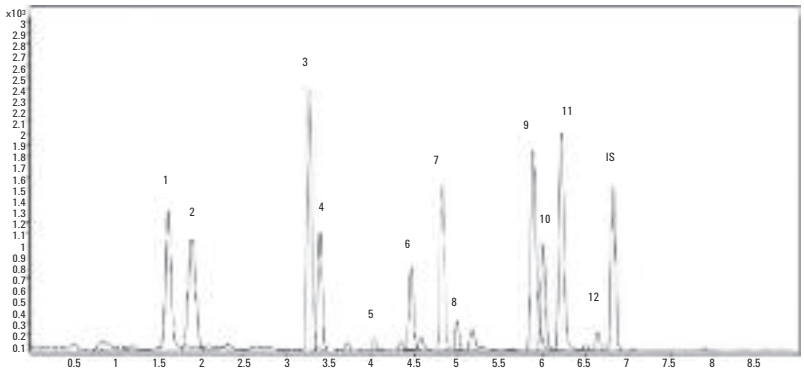
Gradient: 5% B in 1 min, 50% B in 3 min, 90% B in 7 min, 90% B in 8 min, 5% B in 8.2 min, 5% B in 9 min

Temperature: 30 °C

MRM chromatograms of 50 ng/g fortified sample processed by EN method.

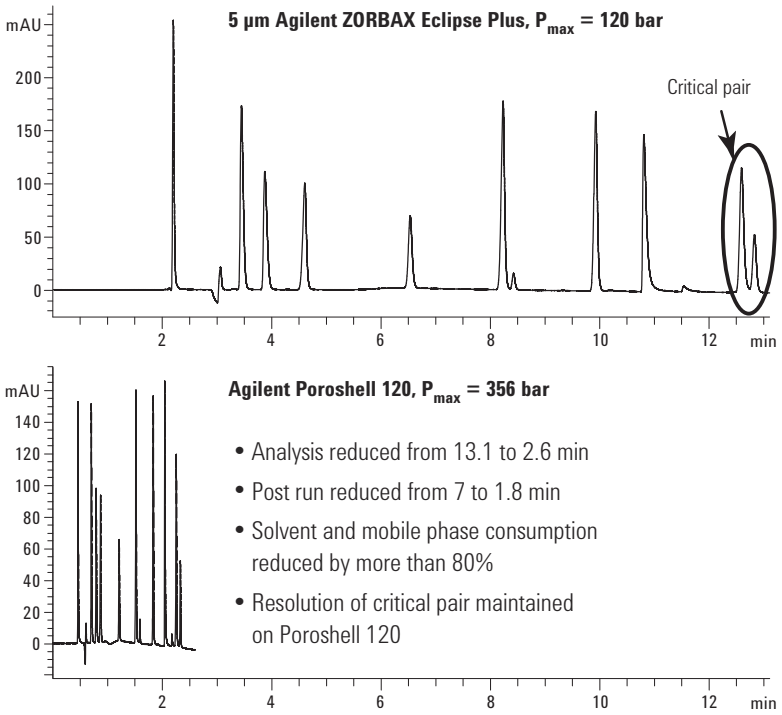
1. Acephate
2. Pymetrozine
3. Carbendazim
4. Thiabendazole
5. Imidacloprid
6. Imazalil
7. Propoxur
8. Carbaryl
9. Cyprodinil
10. Ethoprophos
11. Penconazole
12. Kresoxim-methyl

IS TPP



NEW!

An overlay of the original ZORBAX Eclipse Plus 5 µm method and Agilent Poroshell 120 method. All 11 peaks on Poroshell 120 are resolved by the time the first peak elutes on the original 5 µm ZORBAX Eclipse Plus method



- Analysis reduced from 13.1 to 2.6 min
- Post run reduced from 7 to 1.8 min
- Solvent and mobile phase consumption reduced by more than 80%
- Resolution of critical pair maintained on Poroshell 120

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	1.000 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 12.0 min
Temperature:	30 °C

Column:	**Poroshell 120 EC-C18 695975-302 3.0 x 100 mm, 2.7 µm**
Mobile Phase:	A: 20 mM ammonium acetate, pH 4.80 B: acetonitrile
Flow Rate:	0.851 mL/min
Gradient:	14% B at t_0, ramp to 52% B in 2.1 min
Temperature:	30 °C

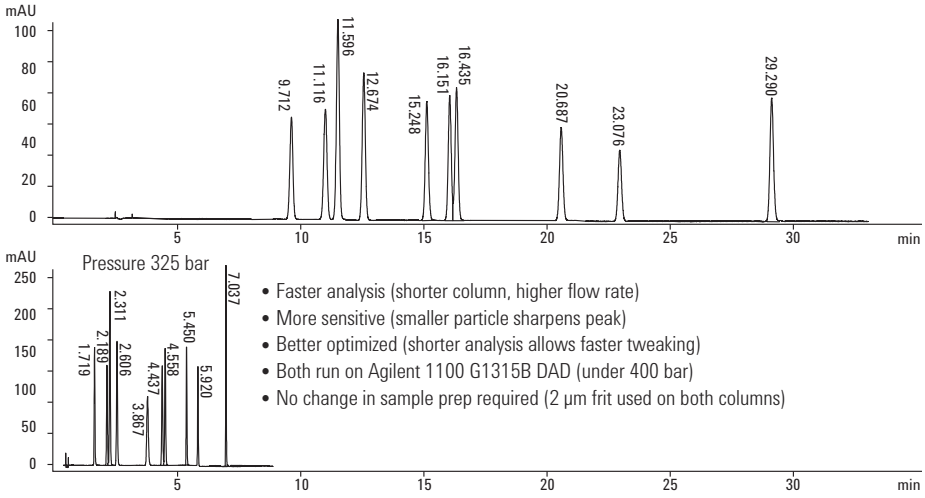
For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Fast analysis of sulfa drugs

Column:	**Eclipse Plus C18 959990-902 4.6 x 250 mm, 5 µm**
Column:	**Poroshell 120 EC-C18 695975-902 4.6 x 100 mm, 2.7 µm**
Gradient:	Formic acid/acetonitrile
Detector:	Agilent 1100 Series LC
Sample:	Ten sulfa drugs

A separation of ten sulfa drugs scaled from an Agilent ZORBAX Eclipse Plus C18 column to an Agilent Poroshell 120 EC-C18 column showing analysis time decreased from 30 min to 8 min using a formic acid/acetonitrile gradient.



- Faster analysis (shorter column, higher flow rate)
- More sensitive (smaller particle sharpens peak)
- Better optimized (shorter analysis allows faster tweaking)
- Both run on Agilent 1100 G1315B DAD (under 400 bar)
- No change in sample prep required (2 µm frit used on both columns)

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Determination of anthocyanins in blueberries

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

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

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

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

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

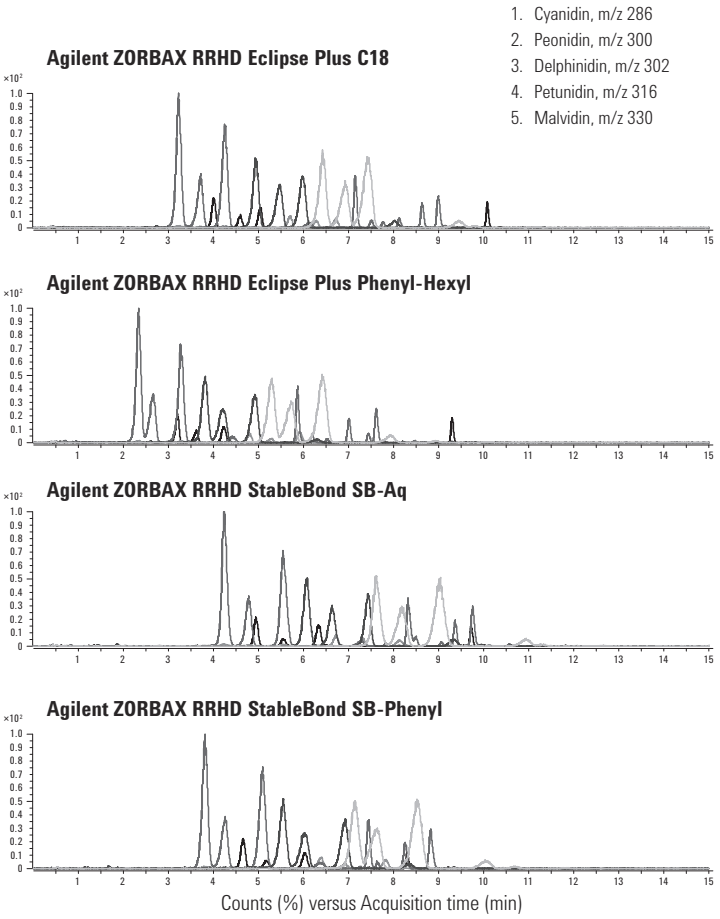
Flow Rate: 0.65 mL

Gradient: 10-50% B in 15 min

Detector: Agilent 1290 Infinity LC

MS Conditions: DAD: Sig = 525, 8 nm; Ref = Off
MS2 Scan: ESI + 200-1000
Scan time: 100 ms, 0.2 amu step
Fragmentor: 180 V
Drying gas: 10 L/min, 350 °C
Nebulizer Pressure: 50 psig
Capillary Voltage: 3500

Sample: 5 µL injection of blueberry extract



Separation of Azo Dyes

Column: **Eclipse Plus Phenyl Hexyl**
959996-912
4.6 x 100 mm, 5 µm

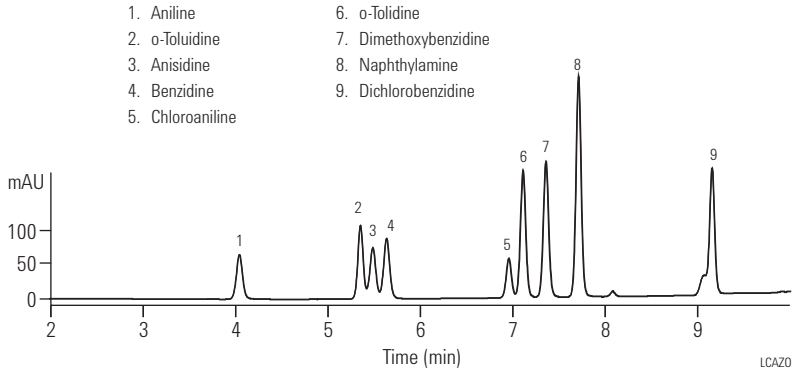
Mobile Phase: A: 10 mM Ammonium Acetate, pH 4.7
B: MeOH

Flow Rate: 1.5 mL/min

Gradient:

Time (Min):	%B:
0	25
5	50

Detector: UV, 254 nm



Anthocyanins from blueberries: High-efficiency high-speed separation

Column A: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

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

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

Mobile Phase: A: 3% Phosphoric acid
B: 100% MeOH

Flow Rate: 1.0 mL/min

Gradient: As shown

Temperature: 30 °C

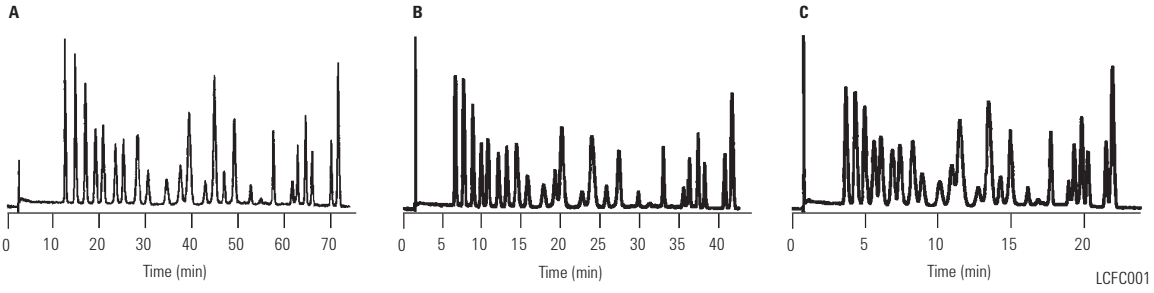
Detector: UV, 525 nm

Sample: Natural anthocyanins

Time	Percent B
0 min	23% B
35 min	26% B
97 min	60% B

Time	Percent B
0 min	23% B
21 min	26% B
58.2 min	60% B

Time	Percent B
0 min	23% B
10.5 min	26% B
29.1 min	60% B



Aromatics II

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

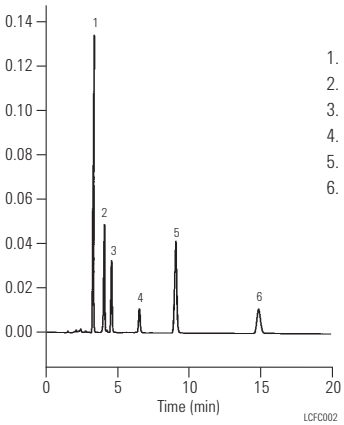
Mobile Phase: H_2O: MeOH, 40:60

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Aromatic Sample



1. Acetophenone
2. Cinnamaldehyde
3. Eugenol
4. Cinnamaldehyde Impurity
5. Ethyl cinnamate
6. p-Cymene

Aspartame: Metabolites and applications

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

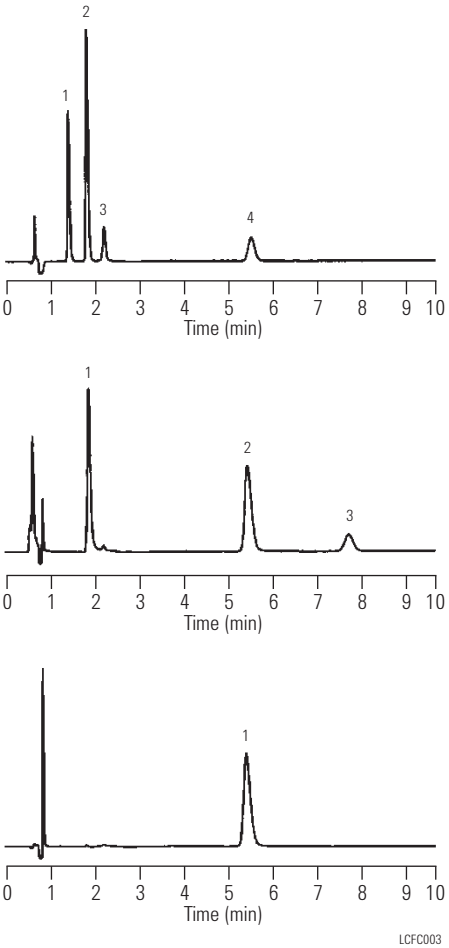
Mobile Phase: 85/15, 0.1% TFA/ACN

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 210 nm

Sample: Aspartame



Aspartame and Its Metabolites

1. Phenylalanine
2. 5-benzyl-3,6-dioxo-2-piperazineacetic acid
3. Aspartic acid-phenylalanine dipeptide
4. Aspartame

Diet Coke

1. Caffeine
2. Aspartame
3. Unknown

Equal Sweetener

1. Aspartame

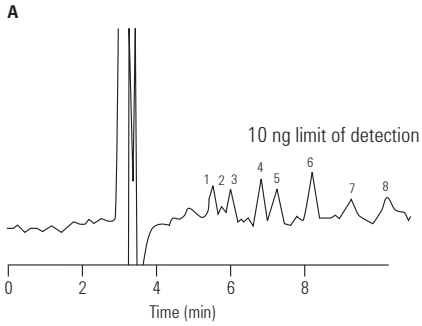
Carbohydrates: Carbohydrate standards

Column: **ZORBAX Carbohydrate Analysis**
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 63% CH_3CN/H_2O
Flow Rate: 0.5 mL/min

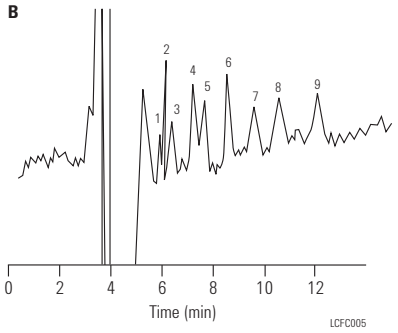
Detector: Agilent RID
Sample: Carbohydrate standard:
A: 25 ng/ L, 1 µL injected
B: 500 pg/ L, 50 µL injected

Carbohydrates: Separation showing high sensitivity



1. Ribose
2. Rhamnose
3. Xylose
4. Fructose
5. Glucose
6. Sucrose
7. Maltose
8. Lactose
9. Raffinose

Sensitivity of high injection volume (50 µL)



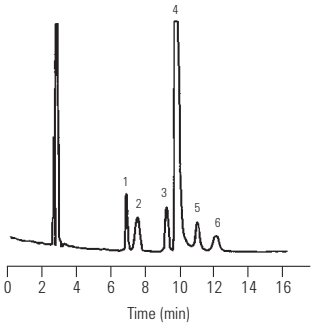
Carbohydrates: Effect of mobile phase strength

Column: **ZORBAX NH2**
880952-708
4.6 x 250 mm, 5 µm

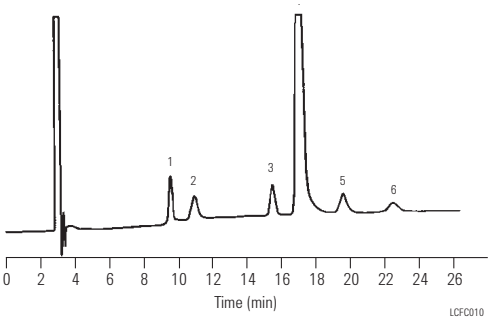
Mobile Phase: ACN/Water, as indicated
Flow Rate: 1.0 mL/min

Temperature: Ambient
Detector: RI
Sample: Mono- and Disaccarides

ACN/H_2O: 70/30



ACN/H_2O: 75/25



1. Fructose
2. Glucose
3. Saccharose
4. Palatinose
5. Trehalulose
6. Isomaltose

Carbohydrates in colas

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

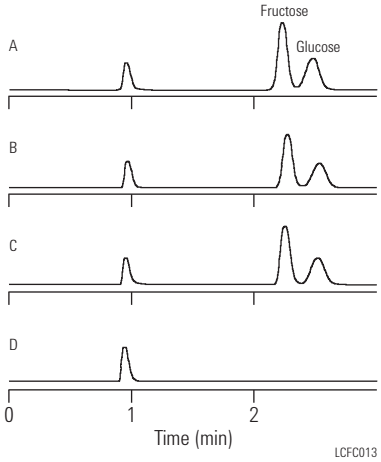
Mobile Phase: 75% ACN:25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: No dilution
A: COLA, Fountain
B: COLA, Can, Brand A
C: COLA, Brand B
D: COLA, Brand B, diet



Carbohydrates: Sugar alcohols

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

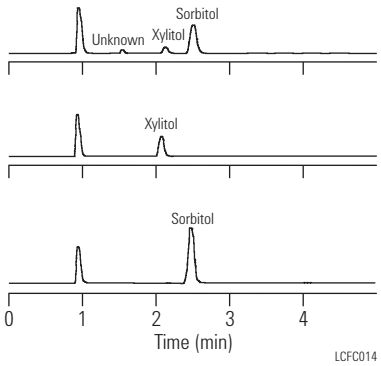
Mobile Phase: 75% ACN:25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Chewing gum, sugar-free



Carbohydrates in juices

Column: **ZORBAX Carbohydrate Analysis 843300-908 4.6 x 150 mm, 5 µm**

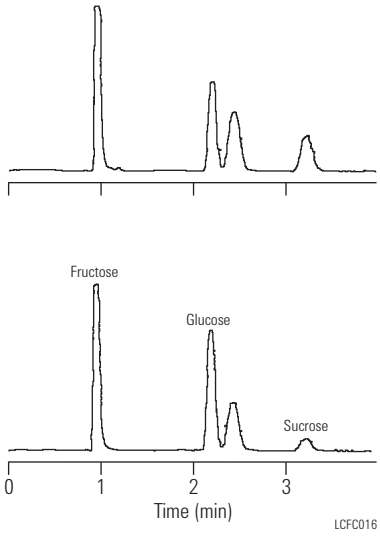
Mobile Phase: 75% ACN/25% H_2O

Flow Rate: 2.0 mL/min

Temperature: 30 °C

Detector: RID

Sample: Diluted to 0.1X in 50:50 ACN:H_2O



Apple Drink
36.8% Fructose
24.9% Sucrose
38.3% Glucose

Apple Juice
58.7% Fructose
9.9% Sucrose
33.4% Glucose

Carbohydrates in milk

Column: **ZORBAX Carbohydrate Analysis**
843300-908
4.6 x 150 mm, 5 µm

Mobile Phase: 75% ACN/25% H_2O

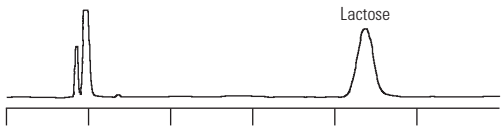
Flow Rate: 2.0 mL/min

Temperature: 30 °C

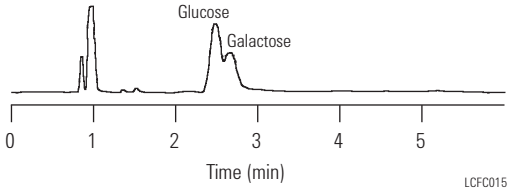
Detector: RID

Sample: Partitioned between CH_3Cl_2: H_2O

Milk (2%)



100% Lactose-Free Milk



Flavoring agents

Column: **ZORBAX SB-Phenyl**
860975-912
2.1 x 50 mm, 5 µm

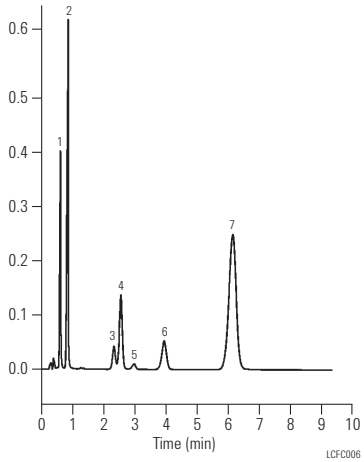
Mobile Phase: 0.3% TFA: ACN, 65:35

Flow Rate: 0.3 mL /min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Cool mint Listerine sample



1. Unknown
2. Benzoic acid
3. Methyl salicylate
4. Carvone
5. Unknown
6. Thymol
7. Anethole

Food colors, FD&C

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

Mobile Phase: A: 0.1% TFA, pH to 4.4 with TEA, B: MeOH

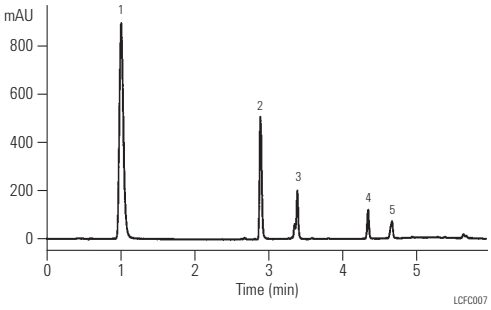
Flow Rate: 1.0 mL/min

Gradient: 17 to 100% B/4 min

Temperature: Ambient

Detector: UV, 254 nm

1. Yellow #5	$C_{16}H_9N_4Na_3O_9S_2$	MW=534
2. Red #40	$C_{18}H_{14}N_2Na_2O_8S_2$	MW=496
3. Blue #1	$C_{37}H_{34}N_2Na_2O_9S_3$	MW=760
4. Propylparaben	$C_{10}H_{12}O_3$	MW=180
5. Red #3	$C_{20}H_{14}Na_2O_5$	MW=878



Neutraceuticals: Extract from green tea

Column: **ZORBAX SB-C8**
863953-906
4.6 x 150 mm, 3.5 µm

Mobile Phase: 75% 0.1% Trifluoroacetic acid: 25% Methanol

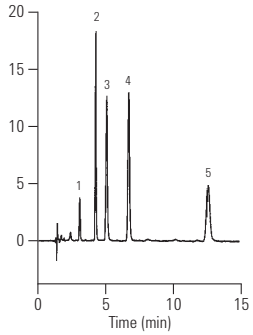
Injection: 1 mL/min

Temperature: 40 °C

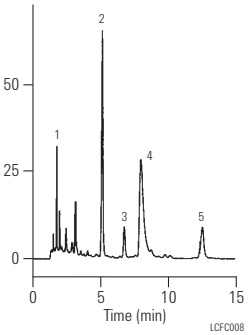
Detector: UV, 280 nm

Sample: Green tea extract, 5 µL

Catechin Mixture



Green Tea Extract



LCFC008

1. Epagallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

Tocopherols by LC/MS with APPI

Column: **Eclipse XDB-C18**
993967-302
3.0 x 150 mm, 5 µm

Mobile Phase: 97% MeOH: 3% 10 mM CH_3COONH_4

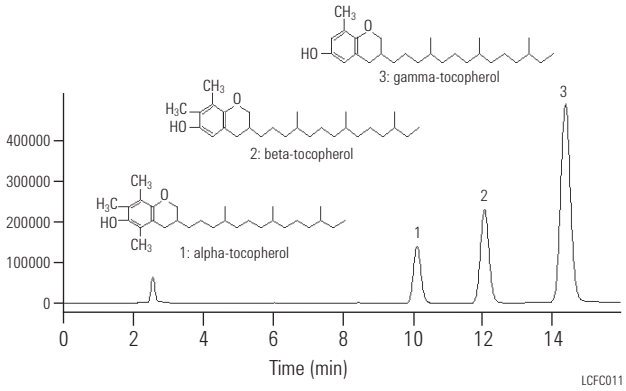
Flow Rate: 0.5 mL/min

Temperature: 40 °C

MS Conditions:

MS:	Agilent 1100MSD SL
Ionization:	APPI (Positive)
Scan range:	m/z 100-500
Vcap:	1500 V
SIM ion:	base peak
Drying gas:	7 L/min at 350 °C
Nebulizer gas:	60 psi
Vaporizer temp:	350 °C
Fragmentor:	140 V
EM gain:	4

Sample Volume: 10 µL



LCFC011

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Sugars in plain and milk chocolate

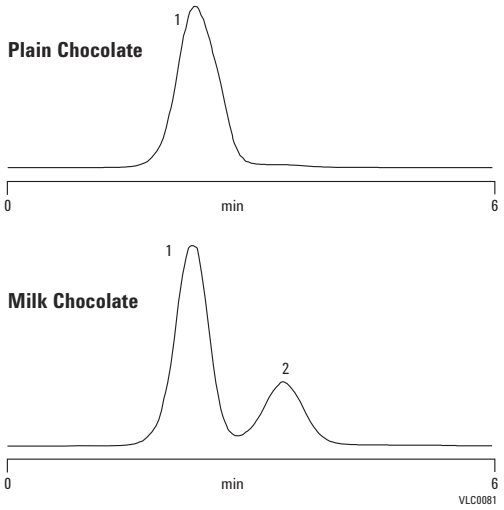
Column: **Hi-Plex Pb**
PL1170-6820
7.7 x 300 mm, 8 µm

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 80 °C

Detector: RI



1. Sucrose
2. Lactose

Sugars

Column: **Hi-Plex K**
PL1170-6860
7.7 x 300 mm, 8 µm

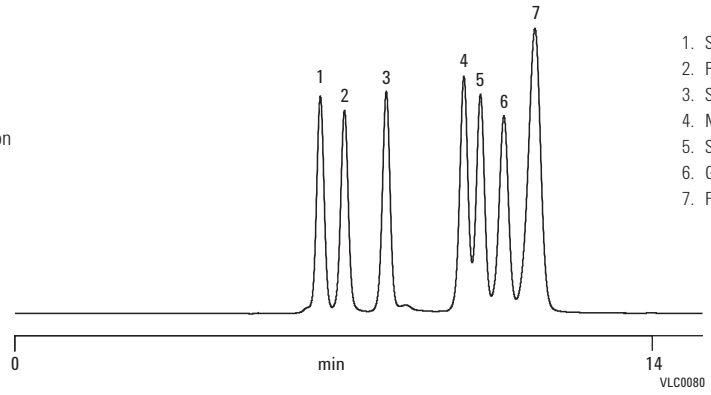
Sample: Sugars mixture (all 10 mg/mL), 20 µL injection

Mobile Phase: Water

Flow Rate: 0.6 mL/min

Temperature: 85 °C

Detector: 356-LC RI



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

Parabens: High speed separation

Column: **ZORBAX SB-C18 Rapid Resolution Cartridge**
833975-902
4.6 x 30 mm, 3.5 µm

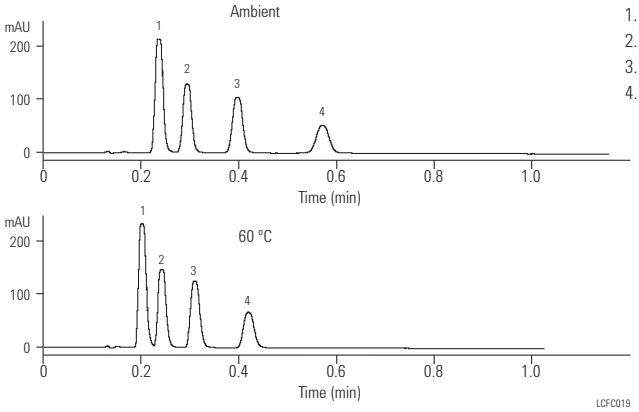
Mobile Phase: 0.1% H_3PO_4:ACN, (50:50)

Flow Rate: 2 mL/min

Temperature: Top: ambient, bottom: 60 °C

Detector: UV, 254 nm with standard flow cell (13 µL)

Sample: Parabens, 1 µL



1. Methylparaben
2. Ethylparaben
3. Propylparaben
4. Butylparaben

Separation of vitamin D2/D3

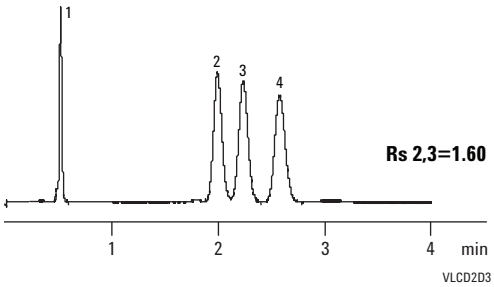
Column: **Eclipse PAH**
959941-918
4.6 x 50 mm, 1.8 µm

Mobile Phase: 92% MeOH, 8% water

Flow Rate: 2 mL/min

Temperature: 40 °C

Detector: 325 nm for VA/280 nm for VD and VE



1. Vitamin A
2. Vitamin D2
3. Vitamin D3
4. Vitamin E (a-VE)

Fat-soluble vitamins on ZORBAX Eclipse XDB-C8

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

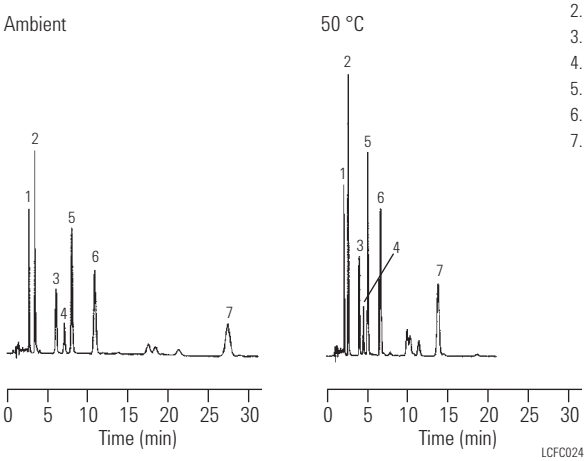
Mobile Phase: 5/95 Water/MeOH

Flow Rate: 1.0 mL/min

Temperature: A: Ambient
B: 50 °C

Detector: UV, 280 nm

Sample: Fat-soluble vitamins



1. Retinol
2. Retinol acetate
3. Vitamin D3
4. γ-Tocopherol
5. α-Tocopherol
6. Tocopherol acetate
7. Retinol palmitate

Water-soluble vitamins

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

Mobile Phase: A: 50 mM Sodium Phosphate, pH 2.5/MeOH (90/10)
B: 50 mM Sodium Phosphate, pH 2.5/MeOH (10/90)

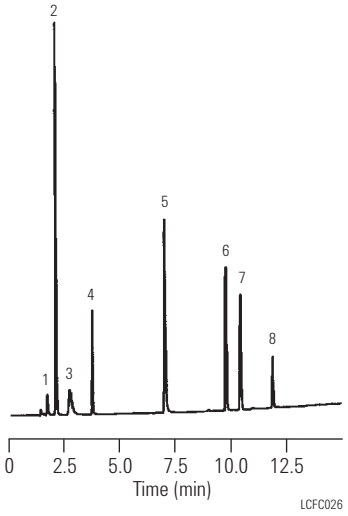
Flow Rate: 1.0 mL/min

Gradient: 0-70% B in 18 min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. B_1-Thiamine
2. Vitamin C
3. B_3-Niacin
4. B_6-Pyridoxine
5. Pantothenic acid
6. Folic acid
7. B_{12}-Cyanocobalamin
8. B_2-Riboflavin

Water-soluble vitamins: High speed separation using ion-pairing

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

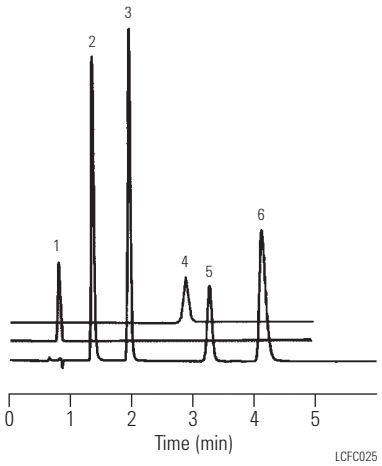
Mobile Phase: 10 mM Hexane Sulfonate with 0.1% Phosphoric Acid: MeOH (74:26)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 245 nm

Sample: Water-soluble vitamins



1. Vitamin C
2. B_3-Niacin
3. B_6-Pyridoxine
4. Folic acid
5. B_2-Riboflavin
6. B_1-Thiamine

Water-soluble vitamins using the USP 23 method

Column: **ZORBAX SB-C18**
880975-902
4.6 x 250 mm, 5 µm

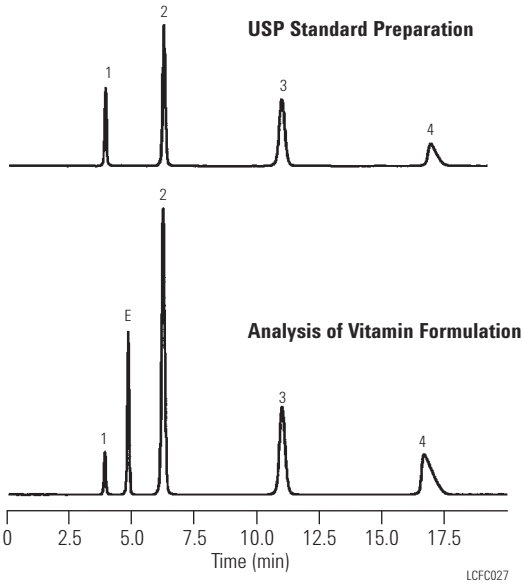
Mobile Phase: 7.2 mM Hexane Sulfonate/MeOH/Acetic Acid (73/27/1) (ratio to 101)

Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: UV, 280 nm

Sample: Water-soluble vitamins



1. B_3-Niacin
2. B_6-Pyridoxine
3. B_2-Riboflavin
4. B_1-Thiamine
E. Excipent

Water-soluble B vitamins separated on ZORBAX SB-Aq

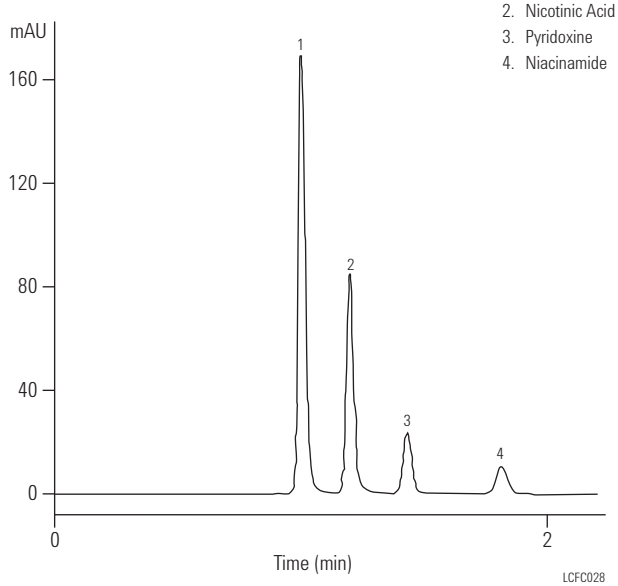
Column: **ZORBAX SB-Aq**
883975-914
4.6 x 150 mm, 5 µm

Mobile Phase: 5% MeOH/95% water (0.1% TFA)

Flow Rate: 2.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm



1. Thiamine
2. Nicotinic Acid
3. Pyridoxine
4. Niacinamide

Sunscreen ingredients: Perform conventional, fast and ultra-fast separations on the same column family

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

Column B: **Eclipse XDB-C18**
961967-902
4.6 x 100 mm, 3.5 µm
4 µL inj

Column C: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm
2 µL inj

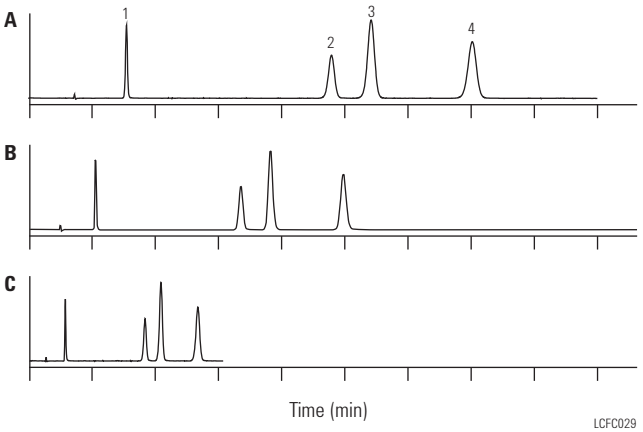
Mobile Phase: A: 15% water
B: 85% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Sunscreens



1. 2-hydroxy-4-methoxybenzophenone
2. Padimate O
3. 2-ethylhexyl trans-4-methoxycinnamate
4. 2-ethylhexyl salicylate

Fast vitamin E analysis on Rapid Resolution HT

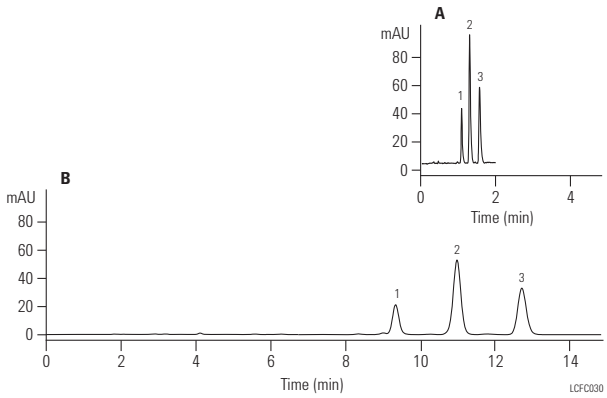
Column A: **Eclipse XDB-C18**
927975-902
4.6 x 50 mm, 1.8 µm

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

Mobile Phase: A: 5% water
B: 95% MeOH

Flow Rate: 3 mL/min, 1 mL/min

Temperature: Ambient



1. α-tocopherol
2. β-tocopherol
3. γ-tocopherol

Theobromine in beverages

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

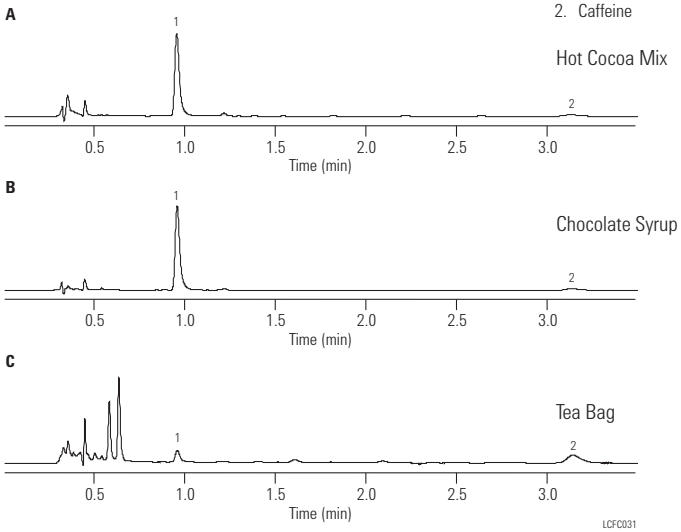
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm, flow cell 2 µL,
3 mm flow path

Sample: Theobromine



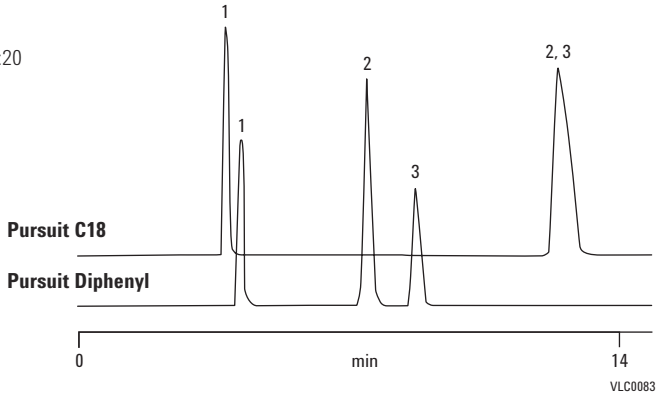
1. Theobromine
2. Caffeine

Benzoic acid/sorbic acid

Mobile Phase: 0.1% formic acid in water:
0.1% formic acid in MeCN, 80:20

Flow Rate: 0.7 mL/min

Detector: UV, 254 nm



1. p-Aminobenzoic acid
2. Sorbic acid
3. Benzoic acid

Quantification and qualification of vitamin C and citric acid in fresh grapefruit juice

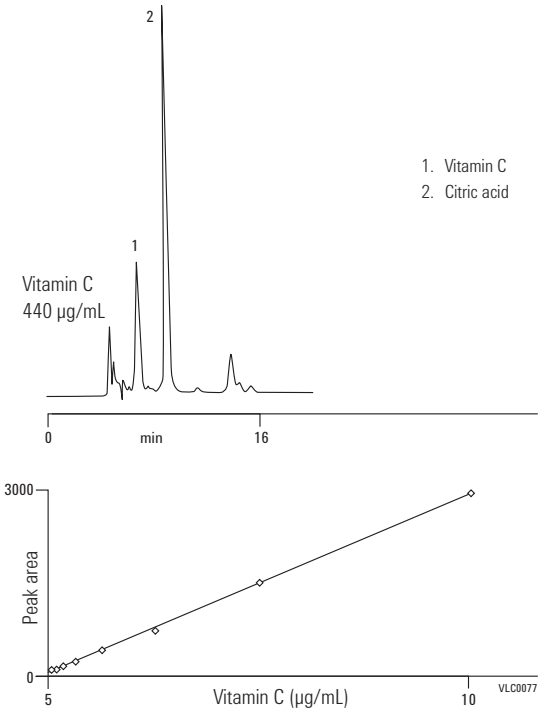
Column: PLRP-S 100Å
PL1512-5500
4.6 x 250 mm, 5 µm

Sample: Diluted 1:50 in eluent

Mobile Phase: 0.2M NaH_2PO_4, pH 2.14

Flow Rate: 0.5 mL/min

Detector: UV, 220 nm



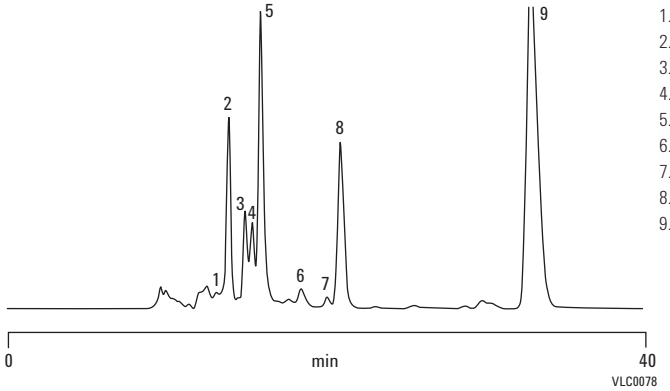
1. Vitamin C
2. Citric acid

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Rose wine

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

Mobile Phase: 0.004M H_2SO_4
Flow Rate: 0.4 mL/min
Pressure: 13 bar
Temperature: 75 °C
Detector: RI

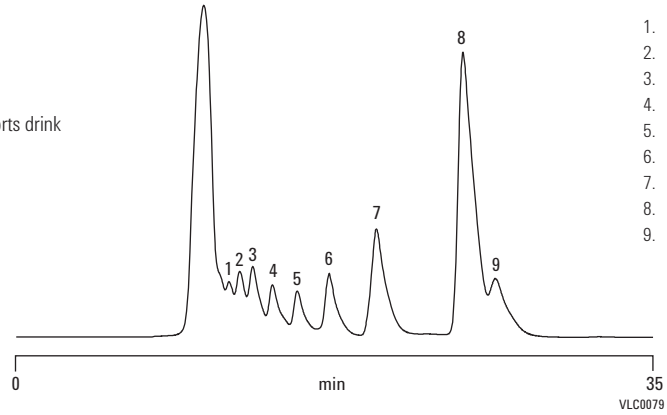


1. Citric acid
2. Tartaric acid
3. Glucose
4. Malic acid
5. Fructose
6. Succinic acid
7. Lactic acid
8. Glycerol
9. Ethanol

Sports drink

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

Sample: High energy orange flavor non-carbonated sports drink
Mobile Phase: Water
Flow Rate: 0.3 mL/min
Temperature: 80 °C
Detector: RI

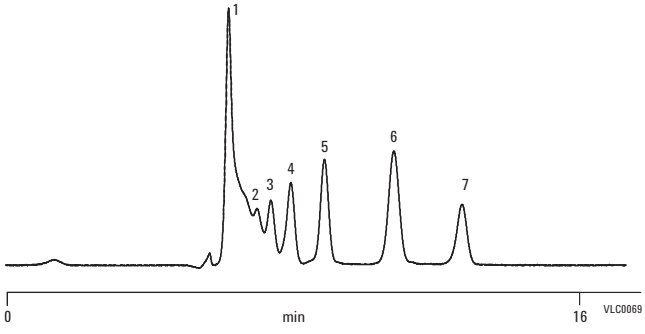


1. Dp8
2. Dp7
3. Dp6
4. Dp5
5. Dp4
6. Dp3
7. Dp2
8. Dp1 (Glucose)
9. Fructose

Oligosaccharides

Column: **Hi-Plex Ca (Duo)**
PL1F70-6850
6.5 x 300 mm, 8 µm

Mobile Phase: DI water
Flow Rate: 0.5 mL/min
Temperature: 90 °C
Detector: RI



1. Higher MW sugars
2. DP5
3. DP4
4. DP3
5. DP2
6. DP1
7. Fructose

Pharmaceutical Applications

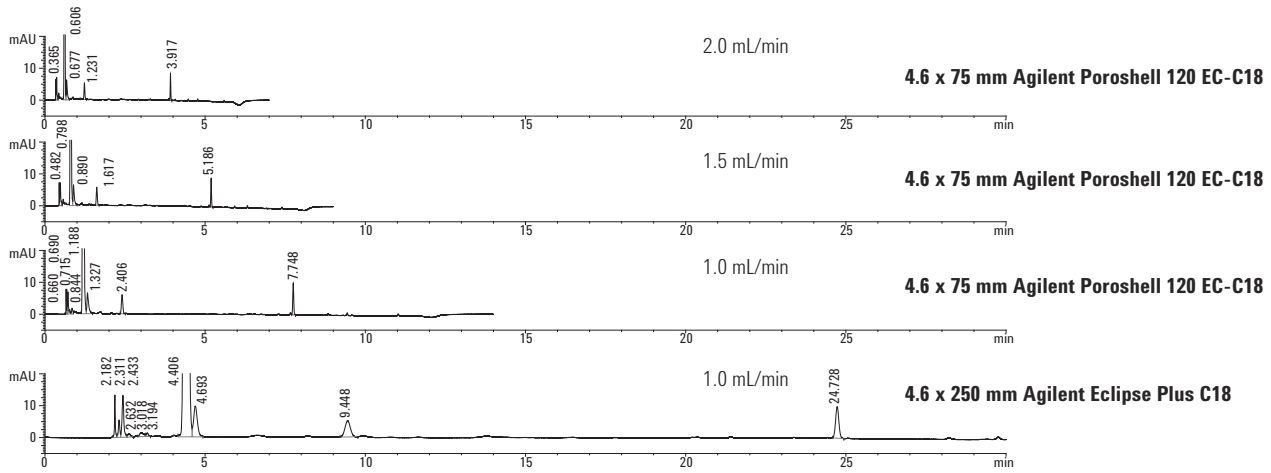
NEW!

Fast analysis of cefepime and related impurities

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

Column: **Eclipse Plus C18**
959990-902
4.6 x 250 mm, 5 µm

Detector: Agilent 1200 Infinity Series



NEW!

Naproxen analysis

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

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

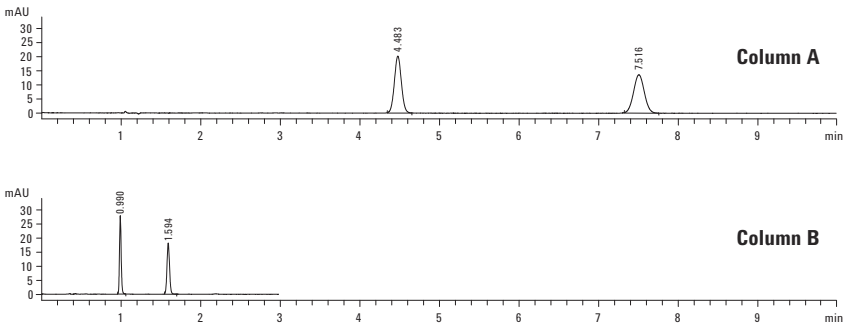
Mobile Phase: 50:49:1 MeCN:H_2O:Glacial acetic acid

Flow Rate: 1.2 mL/min

Injection: Column A: 20 µL
Column B: 6.7 µL

Injection: Naproxen

Method requirement N > 4000 Rs better than 11.5



4-fold reduction in analysis time for this method when transferring to Poroshell 120.

NEW!

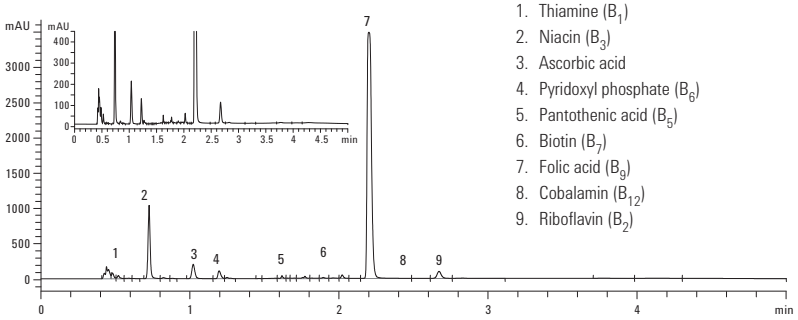
Analysis of water soluble vitamins in multivitamin tablets

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

Flow Rate: 1.5 mL/min

Gradient: 0 min-1% B, 0.5 min-12% B, 0.52 min-30% B, 3.5 min-30% B, 4.5 min-1% B

Injection: 5 µL



1. Thiamine (B_1)
2. Niacin (B_3)
3. Ascorbic acid
4. Pyridoxyl phosphate (B_6)
5. Pantothenic acid (B_5)
6. Biotin (B_7)
7. Folic acid (B_9)
8. Cobalamin (B_{12})
9. Riboflavin (B_2)

NEW!

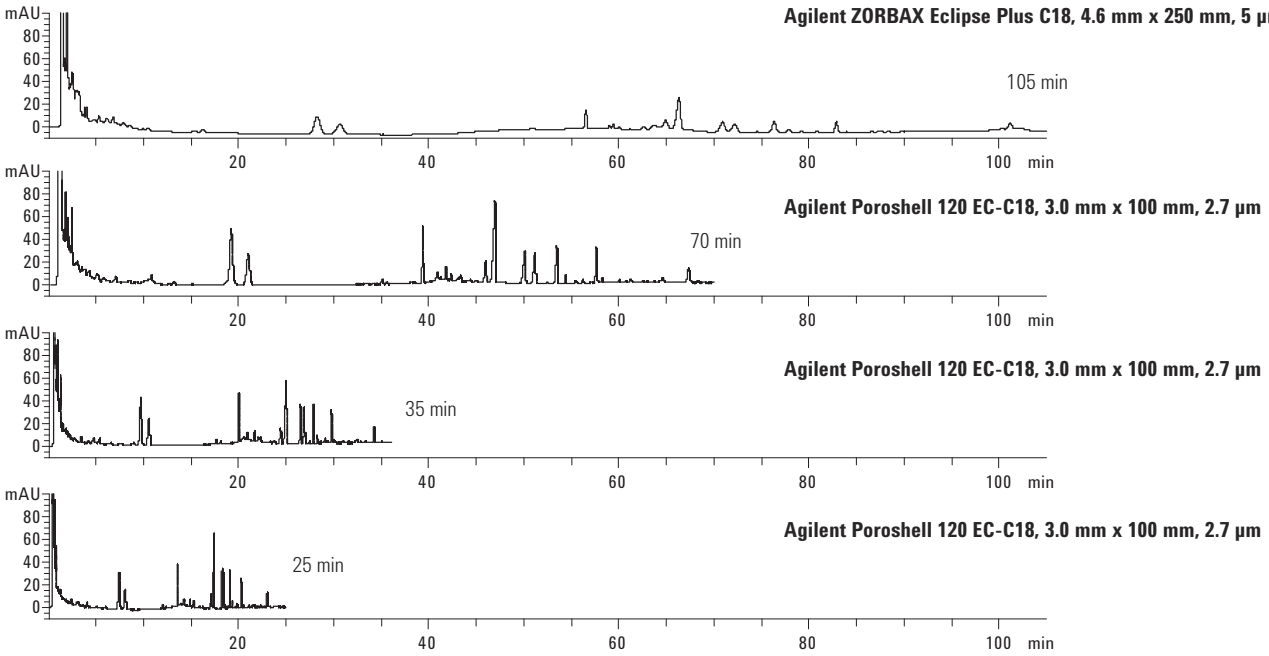
Fast method for ginseng analyses scaled from a traditional method

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

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

Detector: 1200 Infinity Series

Sample: Ginsenoside



Agilent ZORBAX Eclipse Plus C18, 4.6 mm x 250 mm, 5 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

Agilent Poroshell 120 EC-C18, 3.0 mm x 100 mm, 2.7 µm

NEW!

Separation of 8 steroids

Column A: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

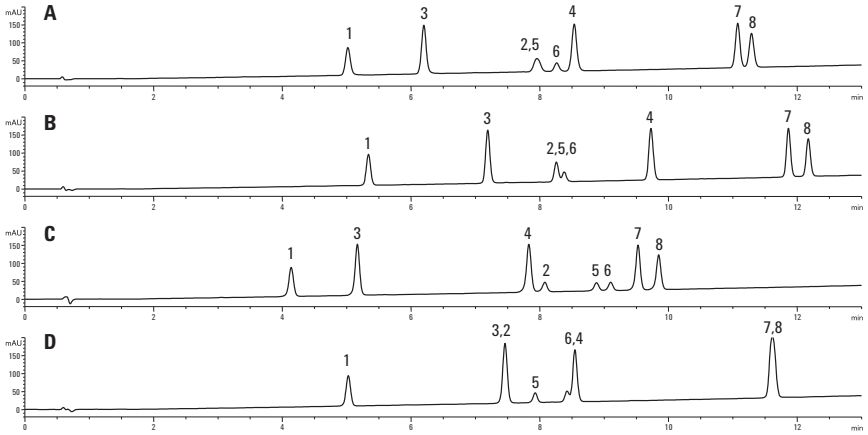
Column D: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Mobile Phase: 0.1% formic acid in both water and MeOH

Flow Rate: 0.4 mL/min, 25 ºC, 2.1 x 100 mm 40 ºC

Gradient: 40-80% MeOH in 14 min

1. Hydrocortisone
2. β-Estradiol
3. Androstadiene 3,17 dione
4. Testosterone
5. Ethynylestradiol
6. Estrone
7. Norethindrone acetate
8. Progesterone



NEW!

Mixture of beta blockers

Column A: **Poroshell 120 Bonus RP**
685775-901
2.1 x 100 mm, 2.7 µm

Column B: **Poroshell 120 Phenyl-Hexyl**
695775-912
2.1 x 100 mm, 2.7 µm

Column C: **Poroshell 120 EC-C18**
695775-902
2.1 x 100 mm, 2.7 µm

Column D: **Poroshell 120 SB-C18**
685775-902
2.1 x 100 mm, 2.7 µm

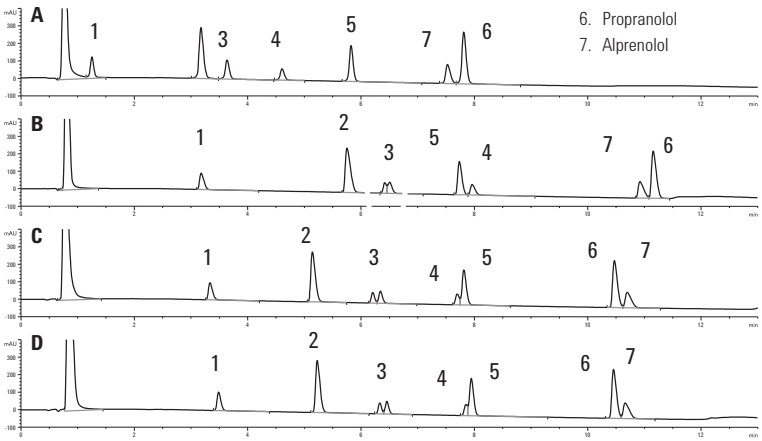
Mobile Phase: 10 mM pH 3.8 NH_4HCO_2, methanol

Flow Rate: 0.35 mL/min

Gradient: 90% B to 30% B over 12 min

* Nadolol is isobaric and elutes as two peaks.

1. Atenolol
2. Pindolol
3. Nadolol
4. Metoprolol
5. Acebutolol
6. Propranolol
7. Alprenolol



NEW!

Several ZORBAX RRHD 1.8 µm selectivities facilitate method development

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

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

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

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

Mobile Phase: A: H_2O
B: CH_3CN, each with 0.1% HCOOH

Detector: Agilent 1290 Infinity LC with an
Agilent 6410 Triple Quadrupole Mass Spectrometer

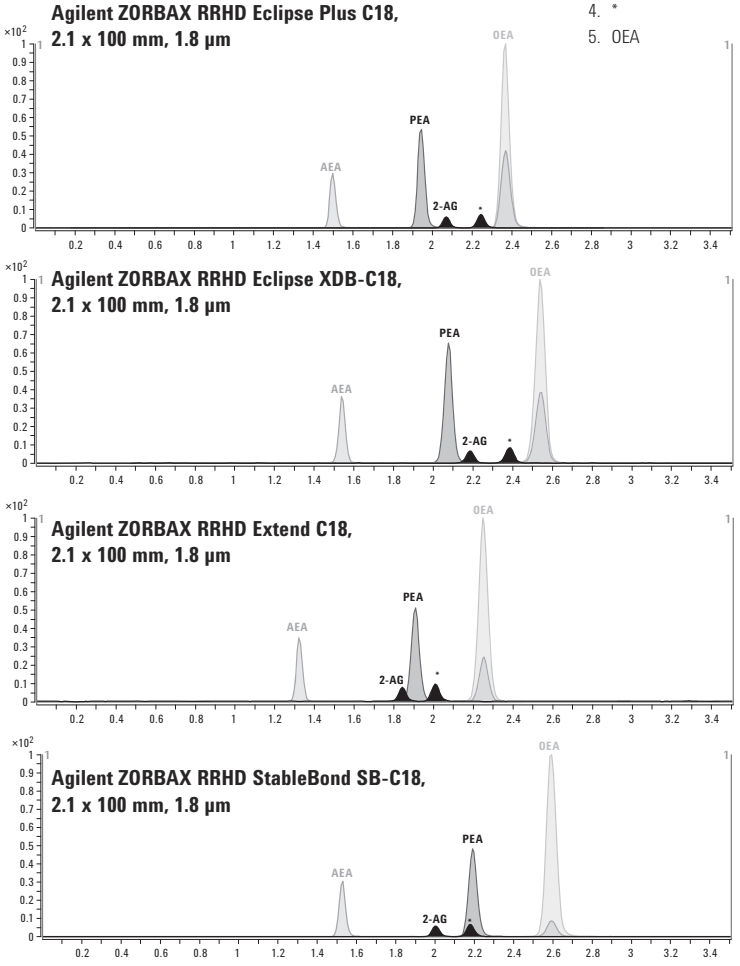
MS Conditions: TCC: 30 °C
MS Source: Electrospray AP-ESI
Drying-gas temperature and flow: 325 °C, 12 L/min
Nebulizer gas pressure: 35 psi
Capillary voltage: 3000 V

Sample: Four endocannabinoid fatty amides:
Arachidonoylglycerol (AEA)
2-Arachidonoylglycerol (2-AG)
Palmitoylethanolamide (PEA)
Oleoylethanolamide (OEA)

*** The second black peak is an impurity, believed to be 1,3-arachidonolyglycerol, a rearrangement of 2-AG**

The selectivity of four Agilent ZORBAX RRHD C18 columns is compared using a method for endocannabinoids.

1. AEA
2. PEA
3. 2-AG
4. *
5. OEA



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Fast analysis 11 common compounds found in analgesics

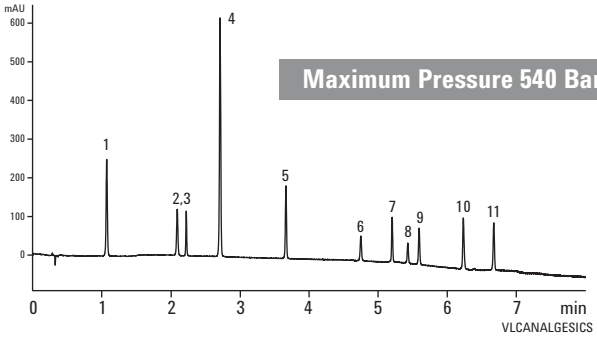
Column: **Poroshell 120 EC-C18**
695975-902
4.6 x 100 mm, 2.7 µm

Mobile Phase: A : Water + 0.1% formic acid
B: ACN

Flow Rate: 3.5 mL/min

Temperature: 40 °C

Detector: DAD 254 nm



Maximum Pressure 540 Bar

1. Acetaminophen
2. Caffeine
3. 2-Acetamidophenol
4. Acetamide
5. Phenacetin
6. Sulindac
7. Piroxicam
8. Tolmetin
9. Ketoprofen
10. Diflusinal
11. Diclofenac

Faster analysis of USP Method for simvastatin tablet

Column A: **Eclipse Plus C18**
959990-902
4.6 x 250 mm, 5 µm

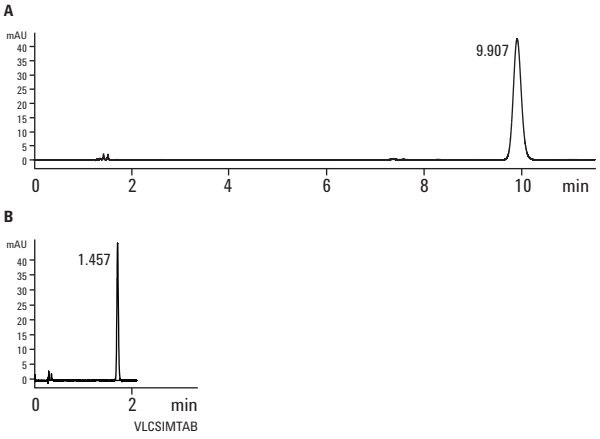
Column B: **Poroshell 120 EC-C18**
697975-902
4.6 x 75 mm, 2.7 µm

Mobile Phase: 65% CH_3CN,
35% 3.9 g/L NaH_2PO_4 (pH 4.5)

Flow Rate: 1.5 mL/min for 5 µm column
2.8 mL/min for 2.7 µm Poroshell 120 column

Temperature: 45 °C

Detector: DAD Sig = 238, 8
Ref = 360, 100 nm



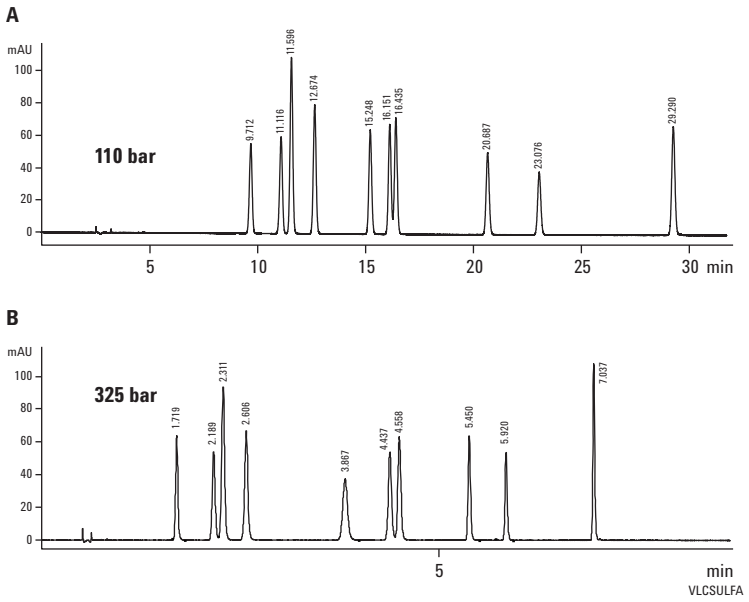
	USP Requirement	5 µm (1.5 mL/min)	2.7 µm (2.8 mL/min)
T_R	N/A	9.907	1.457
k'	> 3.0	5.962	5.122
N	> 4500	16939	14439
T_f	< 2.0	1.09	1.10

Faster separation of sulfa drugs

		Time	%B
Column A:	**Eclipse Plus C18**	0	8
	959990-902	33	33
	4.6 x 250 mm, 5 µm	35	33
Column B:	**Poroshell 120 EC-C18**	**Time**	**%B**
	695975-902	0	8
	4.6 x 100 mm, 2.7 µm	12	33
		13.2	33

Mobile Phase: A: 0.1% formic acid in Water
B: 0.1% formic acid in ACN

Flow Rate: 1 mL/min



Separation of pharmaceutical cardiac drugs

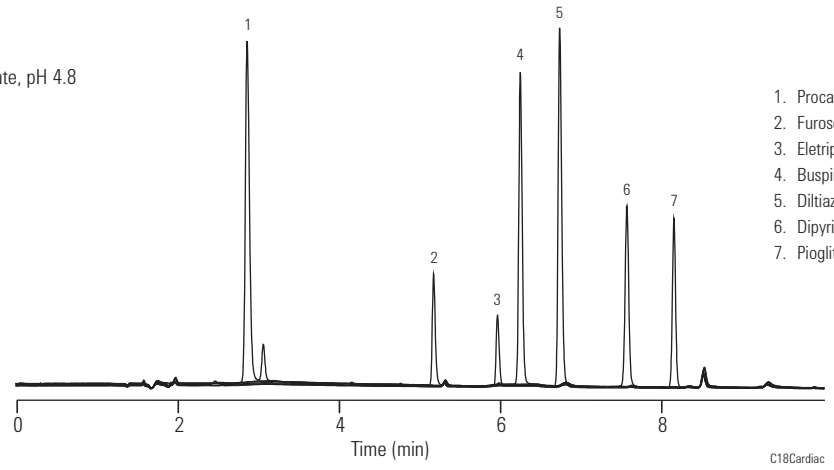
Column: **Eclipse Plus C18**
959996-902
4.6 x 100 mm, 5 µm

Mobile Phase: A: 20 mM Ammonium Acetate, pH 4.8
B: ACN

Flow Rate: 1 mL/min

Gradient: 10-90% in 10 min

Detector: UV, 254 nm



1. Procainamide
2. Furosemide
3. Eletriptan
4. Buspirone
5. Diltiazen
6. Dipyridamole
7. Pioglitazone

Fast and ultra-fast analysis of basic compounds

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

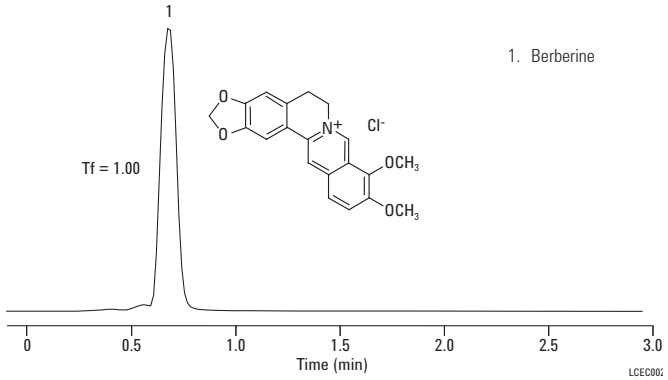
Mobile Phase: A: 50% 8 mM K_2HPO_4, pH 7
B: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Berberine, 0.4 mg/mL, 2 µL



1. Berberine

Xanthines: Higher resolution, same selectivity with RRHT

Column A: **ZORBAX SB-C18**
846975-902
4.6 x 50 mm, 5 µm

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

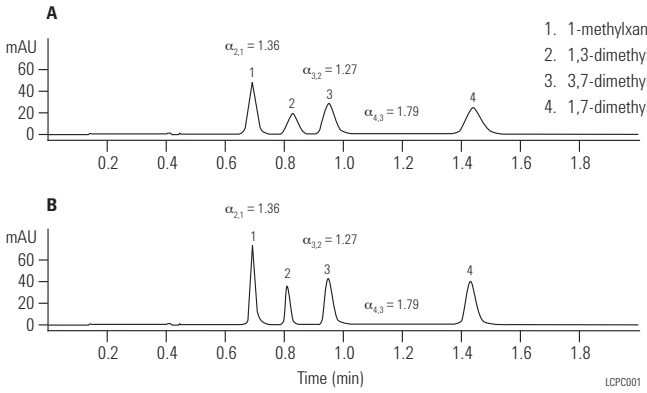
Mobile Phase: A: 92% 0.1% formic acid
B: 8% 0.1% formic acid in ACN

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Xanthines



1. 1-methylxanthine
2. 1,3-dimethyluric acid
3. 3,7-dimethylxanthine (theobromine)
4. 1,7-dimethylxanthine

Antihistamines: Fast separations on RRHT Extend-C18

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

Column B: **ZORBAX Extend-C18**
727975-902
4.6 x 50 mm, 1.8 µm

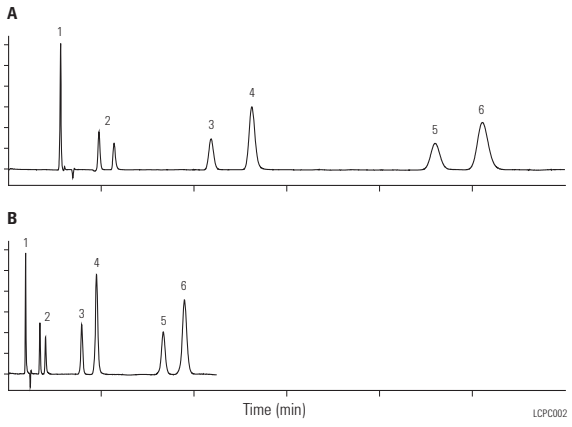
Mobile Phase: A: 30% 50 mM pyrrolidine buffer
B: 70% MeOH

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antihistamines



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

Ibuprofen:
Optimizing selectivity with RRHT Columns

Column A: **SB-C8**
827975-906
4.6 x 50 mm, 1.8 µm

Column B: **Eclipse XDB-C8**
927975-906
4.6 x 50 mm, 1.8 µm

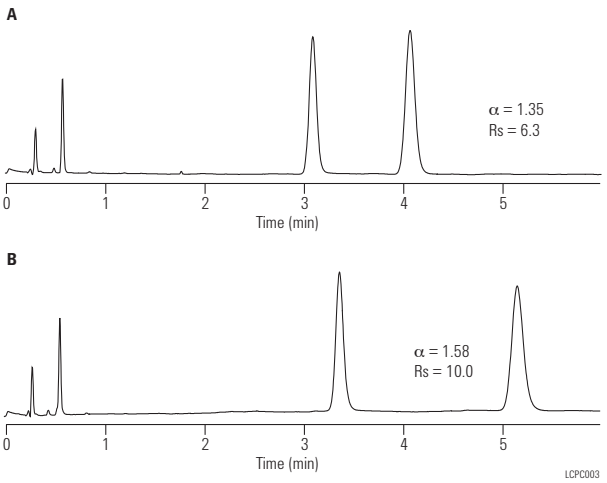
Mobile Phase: A: 63% water
B: 37% acetonitrile + 1.8 mL H_3PO_4

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Ibuprofen oral suspension



1. Benzophenone
2. Ibuprofen

Analgesics

Column: **Pursuit XRs Diphenyl**
A6020150X046
4.6 x 150 mm, 5 µm

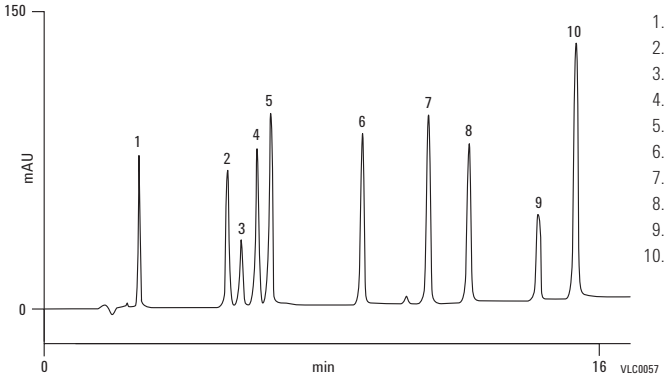
Mobile Phase: A: Water+0.1% HCOOH
B: MeCN+0.1% HCCOH

Gradient: 25-80% B in 20 min

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



1. Acetaminophen
2. Acetanilide
3. Salicylic acid
4. Acetylsalicylic acid
5. Phenacetin
6. Carbamazepine
7. Tolmetin
8. Naproxen
9. Ibuprofen
10. Diclofenac

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Anesthetics, local: Bonded phase selectivity

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

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

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

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

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

Mobile Phase: A: 50 mM NaH_2PO_4 pH 2.5 in 95% H_2O/5% ACN
B: 50 mM NaH_2PO_4 pH 2.5 in 47% H_2O/53% ACN

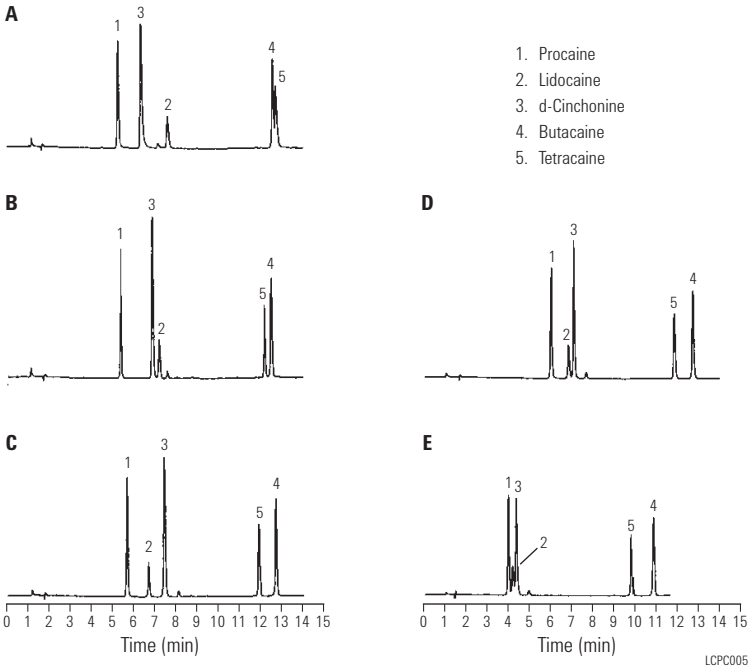
Flow Rate: 1.5 mL/min

Gradient: 0-100% B in 18.8 min

Temperature: 26 °C

Detector: UV, 254 nm

Sample: 10 µL, 10 ug/mL



1. Procaine
2. Lidocaine
3. d-Cinchonine
4. Butacaine
5. Tetracaine

Local anesthetics

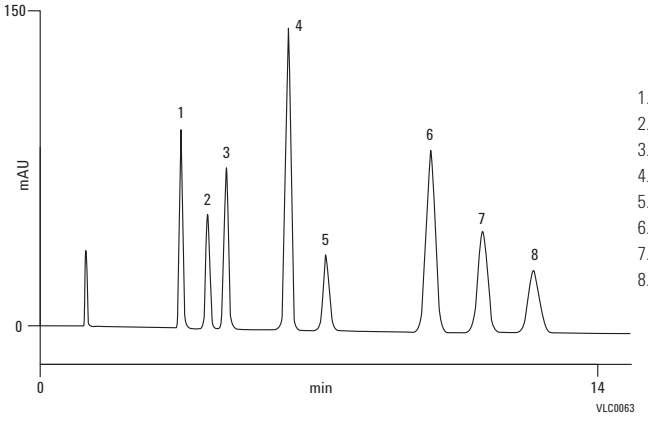
Column: **Pursuit XRs C8**
A6010150X046
4.6 x 150 mm, 5 µm

Mobile Phase: 65:35 MeOH:5 mM NH_4CO_3, pH 10

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 210 nm



1. Benzocaine
2. Procaine
3. Chlorocaine
4. Mepivacaine
5. 4-Hydroxyropivacaine
6. Cocaine
7. Lidocaine
8. Ropivacaine

Antibiotics: High speed separation

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

Mobile Phase: 8.0% acetonitrile/92% 0.1% aqueous TFA

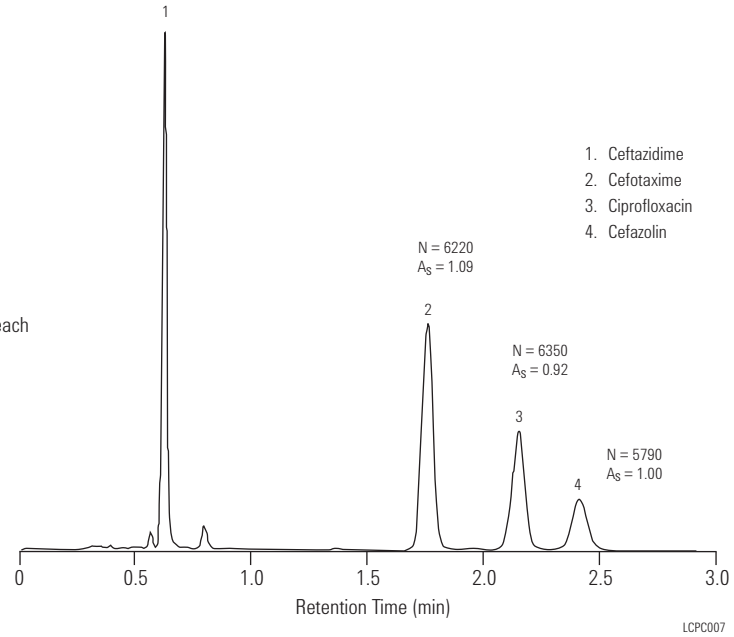
Flow Rate: 3.0 mL/min

Gradient: 45-70% B in 35 min

Temperature: 60 °C

Detector: UV, 260 nm

Sample: 1 µL containing 0.40, 0.36, 0.10 and 0.37 µg each of 1-4 resp.



Antibiotics: Lincomycin and Clindamycin by LC-APCI-MS LC-TIC

Column: **ZORBAX SB-C18 cartridge**
823700-902
2.1 x 30 mm, 1.8 µm

Mobile Phase: Gradient: 15-50% B in 1 min, hold for 1.5 min,
A: 0.2% formic acid pH 2.8
B: ACN + 0.2% formic acid

Flow Rate: 0.5 mL/min

Gradient: Post time: 1.5 min

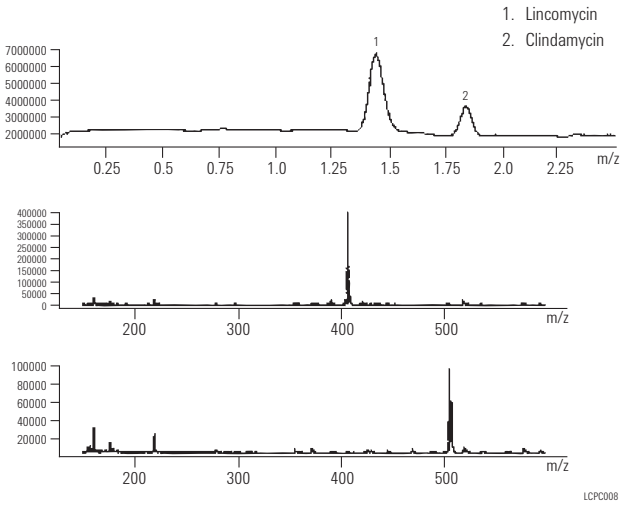
Temperature: Ambient

Detector: APCI, Positive ion

MS Conditions:

Peak width:	0.10 min
Scan:	150-600 Da, step 0.1
Fragmentor:	70
Gas Temp:	350 °C
Vaporizer:	350 °C
Drying gas:	12 L/min
Nebulizer pres:	50 psi
Vcap:	+3000 V
Corona:	4.0 µA

Sample: Antibiotics, 1 µL



Antifungal medications

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

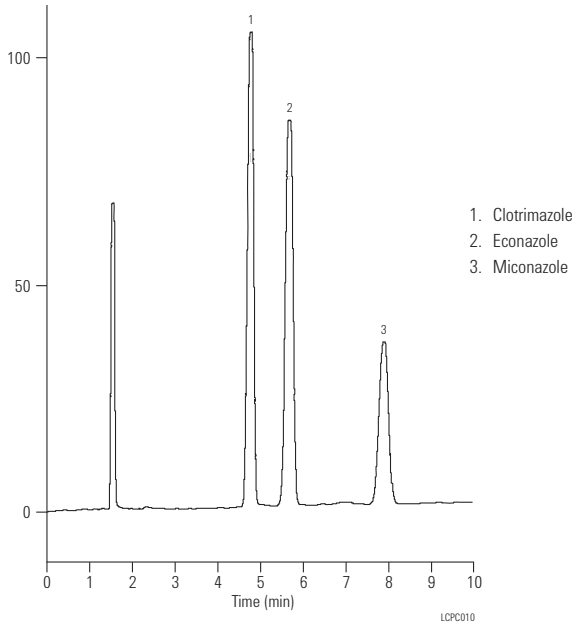
Mobile Phase: 35% 25 mM NaH_2PO_4, Dibasic (pH 6.5 with H_3PO_4): 65% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Antifungals, 2 µL



1. Clotrimazole
2. Econazole
3. Miconazole

Antifungals

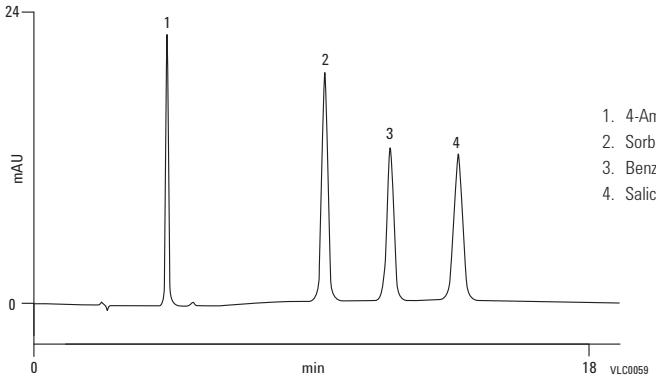
Column: **Pursuit XRs Diphenyl**
A6020150X046
4.6 x 150 mm, 5 µm

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

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm



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

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Analgesics: Non-steroidal anti-inflammatory drugs: Narrow bore separation

Column: **Eclipse XDB-C8**
993700-906
2.1 x 150 mm, 5 µm

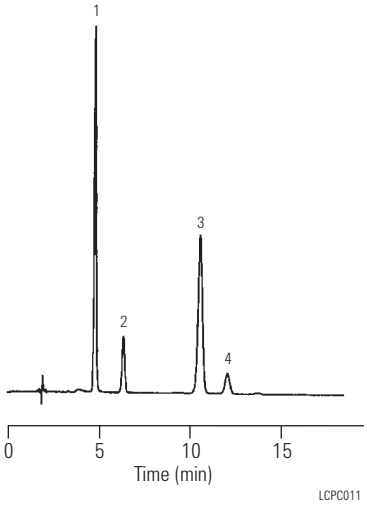
Mobile Phase: 50/50, 25 mM Sodium Phosphate (pH 7.0 with Phosphoric Acid), MeOH

Flow Rate: 0.2 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: 2 µL, 10 ug/mL



NSAID	pK_a
1. Phenacetin	2.2
2. Tolmetin	3.5
3. Phenylbutazone	4.4
4. Fenoprofen	4.5

Separation of small molecule anorectics

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

Column B: **Traditional Alkyl C8 Phase**

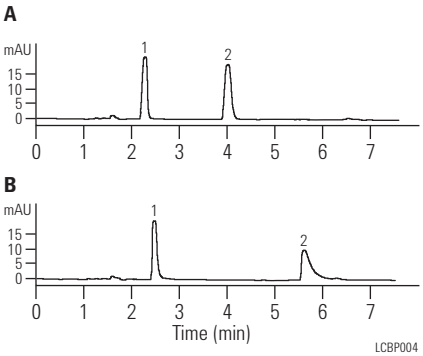
Mobile Phase: 25 mM K_2HPO_4, pH 7.2/MeOH: ACN (50:50), 45/55

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Anorectics "Fen-phen", 5 µL



1. Phentermine
2. Fenfluramine

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Aromatic acids/benzoic acids: Selectivity differences

Column A: **ZORBAX SB-C8**
880975-906
4.6 x 250 mm, 5 µm

Column B: **ZORBAX SB-Phenyl**
880975-912
4.6 x 250 mm, 5 µm

Column C: **ZORBAX SB-CN**
880975-905
4.6 x 250 mm, 5 µm

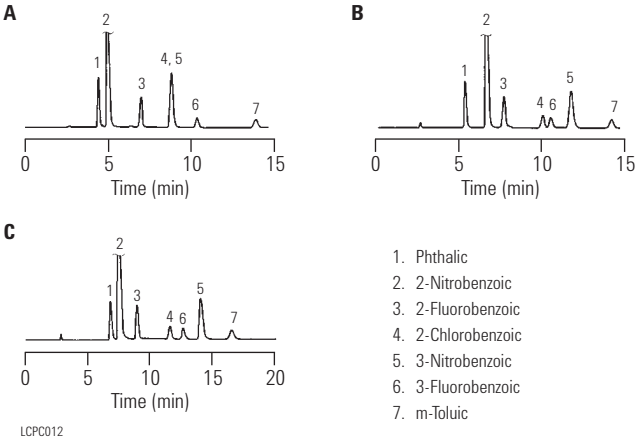
Mobile Phase: 30-45% methanol in 25 mM Na Phosphate, pH 2.5
A: 45% Methanol
B: 40% Methanol
C: 30% Methanol

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Benzoic acids



LCPC012

1. Phthalic
2. 2-Nitrobenzoic
3. 2-Fluorobenzoic
4. 2-Chlorobenzoic
5. 3-Nitrobenzoic
6. 3-Fluorobenzoic
7. m-Toluic

Catecholamines/biogenic amines: Rapid separation using ion-pair reagents

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

Mobile Phase: 0.14 M sodium phosphate,
20 mM EDTA,
0.75 mM octyl sulfonate,
9% methanol pH 3.5

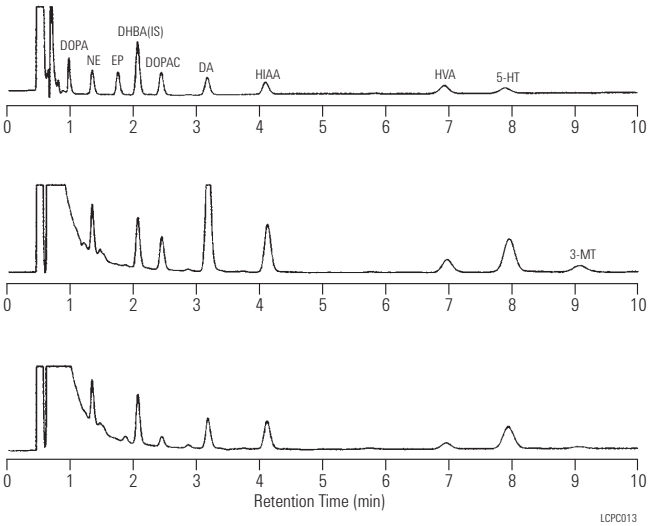
Flow Rate: 1.5 mL/min

Temperature: 26 °C

Detector: 0.75 V vs Ag/AgCl with electro-chemical detection

Sample: 10 µg/mL each standard; volume
20 µL (2 g tissue sample)
A: Standards (2pmol; DHBA 5pmol)
B: Mouse Sriatum
C: Mouse Neocortex

1. DOPA-Dihydroxyphenylalanine
2. DHBA-Dihydroxybenzyl amine
3. DOPAC-Dihydroxyphenyl acetic acid
4. NE-Norepinephrine
5. DA-Dopamine
6. HIAA-Hydroxyindoleaacetic acid
7. EP-Epinephrine
8. HVA-Homovanillic acid
9. 5-HT-Hydroxytryptamine
10. 3-MT-Methaxytyrosine



LCPC013

Chiral ethiazide (diuretic drug) separation

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

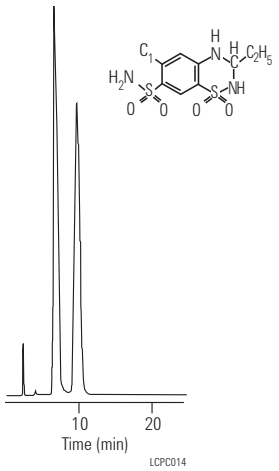
Mobile Phase: 20 mM KH_2PO_4 (pH 4.6)

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg Ethiazide



Chiral separation of fluoxetine enantioners (Prozac)

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

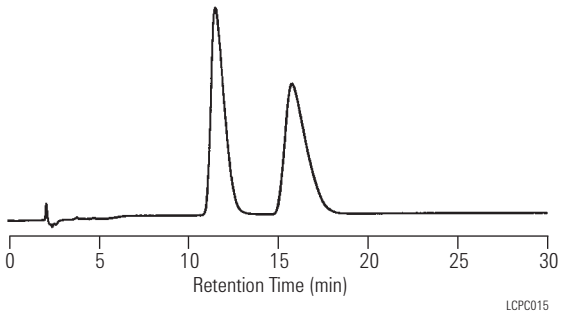
Mobile Phase: 25/75 (v/v) EtOH / 20 mM KH_2PO_4, pH 5.5 (adjusted with NaOH)

Flow Rate: 0.8 mL/min

Temperature: Ambient

Detector: UV, 225 nm

Sample: Mixture fluoxetine (Prozac) enantiomers



Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.

For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Goldenseal and related alkaloids on Rapid Resolution Eclipse XDB-C18

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

Mobile Phase: 68% 30 mM ammonium acetate,
14 mM TEA, pH ~4.85
32% Acetonitrile

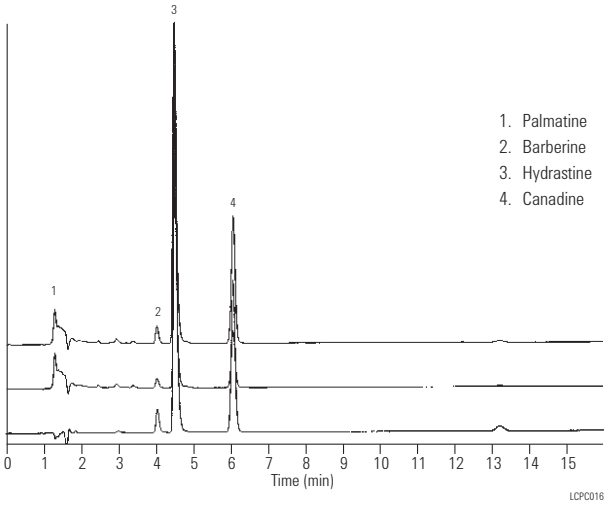
Flow Rate: 1.0 mL/min

Temperature: 30 °C

Detector: 230 nm

Sample: Goldenseal and related alkaloids

Alkaloids, such as the active components in Goldenseal and other related plants, are quickly and accurately separated using isocratic conditions on an Eclipse XDB-C18 Rapid Resolution column.



1. Palmatine
2. Barberine
3. Hydrastine
4. Canadine

Components of green tea separated on Rapid Resolution StableBond SB-C8

Column: **ZORBAX SB-C8**
863953-906
4.6 x 150 mm, 3.5 µm

Mobile Phase: 75% 0.1% TFA : 25% MeOH

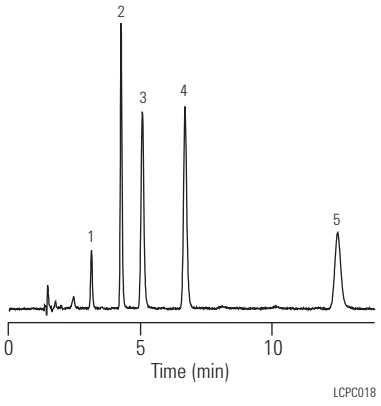
Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: 280 nm

Sample: Green tea

Nutraceuticals, such as the components of green tea, are quickly separated on a StableBond SB-C8 Rapid Resolution column.



1. Epigallocatechin
2. Epicatechin
3. Epigallocatechin gallate
4. Catechol
5. Epicatechin gallate

Chiral separation of hexobarbitol

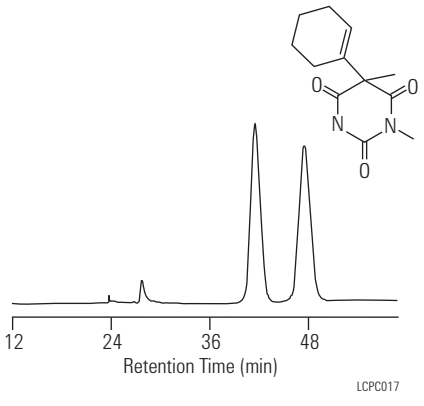
Column: **Chiradex**
79925CB-584
4.0 x 250 mm, 5 µm

Mobile Phase: Methanol/water, 20:80

Flow Rate: 1.0 mL/min

Detector: UV, 220 nm

Sample: Hexobarbitol



Chiral separation of S- and R-Norfluoxetine

Column: **Ultron ES-OVM Chiral**
724111653
4.6 x 250 mm, 10 µm

Mobile Phase: 6/94 (v/v) MeOH / 20 mM KH_2PO_4

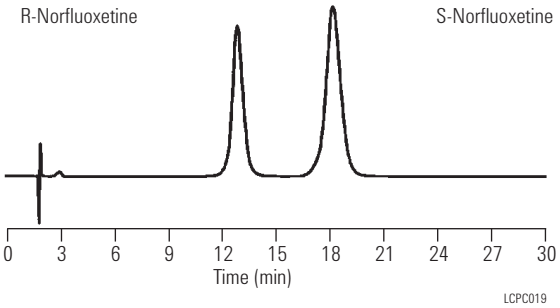
Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 225 nm

Sample: 50 µg/mL of 2:3 mixture R- : S-Norfluoxetine

Courtesy of D.S. Ristry and V.S. Sharp, Eli Lilly and Co.



Chiral separation of salbutamol

Column: **Ultron ES-Pepsin**
822111631A
4.6 x 150 mm, 5 µm

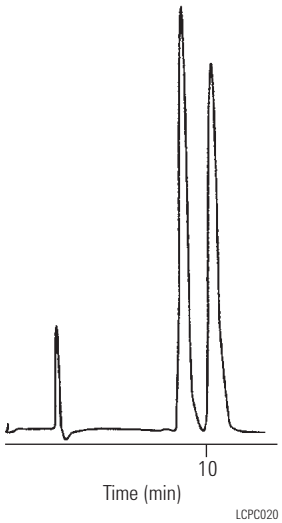
Mobile Phase: 20 mM phosphate buffer, pH 6.0

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm

Sample: 20 µL containing 0.35 µg salbutamol mixture



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

Chiral separation of tolperison enantiomers

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

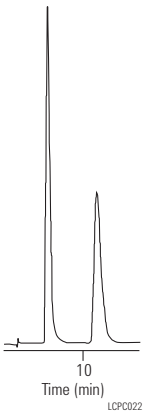
Mobile Phase: 20 mM KH_2PO_4 (pH 5.5), C_2H_5OH (100/4 v/v)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm, 0.04 AUFS

Sample: Tolperison, 5 µL



Chiral separation of atenolol

Column: **Ultron ES-Pepsin**
822111631A
4.6 x 150 mm, 5 µm

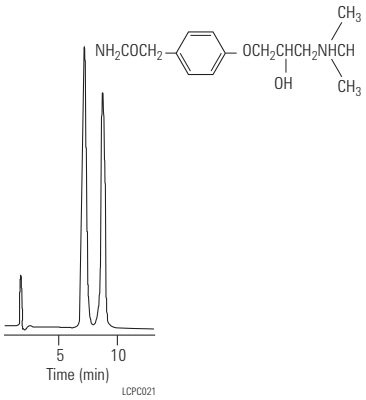
Mobile Phase: 20 mM phosphate buffer, pH 6.0/Ethanol (99/1)

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 220 nm, 0.04 AUFS

Sample: 1.5 µL, 0.25 mg/mL, atenolol racemic mixture



Cocaine and metabolites

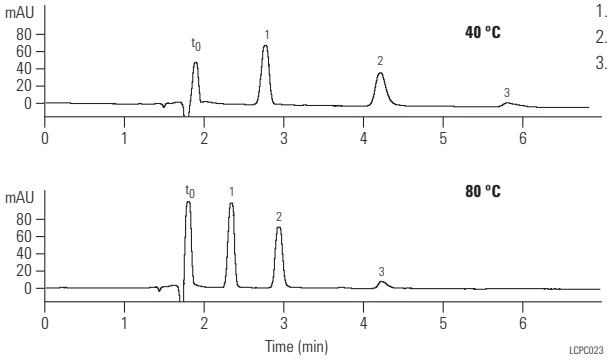
Column: **ZORBAX Rx-SIL**
883975-901
4.6 x 150 mm, 5 µm

Mobile Phase: MeOH: NH_4 Acetate, 25 mM, pH 6 (70:30)

Flow Rate: 1.0 mL/min

Temperature: 40 and 80 °C

Detector: UV, 210 nm



1. Benzoylecgonine
2. Cocaine
3. Ecgonine methylester

Aspirin and cough remedy

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

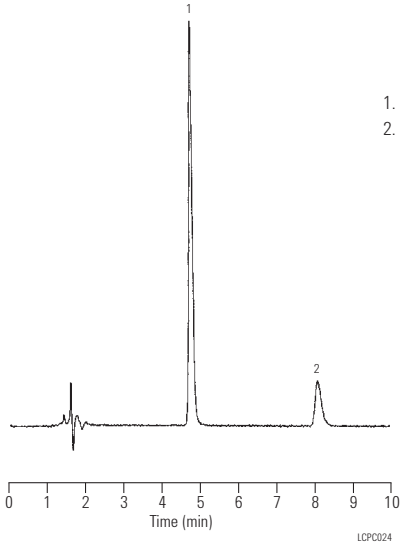
Mobile Phase: (75:25) 25 mM Na_2HPO_4 (pH 3.0): ACN

Flow Rate: 1.0 mL/min

Temperature: 40 °C

Detector: UV, 254 nm

Sample: 5 µL, 10 µg/mL



1. Acetylsalicylic acid
2. Dextromethorphan

Cough formula mixture: Fast and efficient separation

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

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

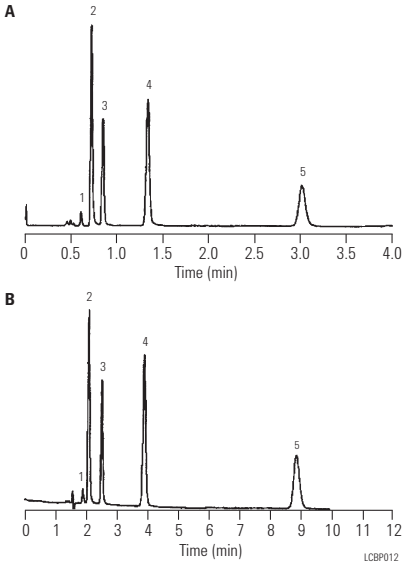
Mobile Phase: 20/80, Acetonitrile/150 mM Na Citrate, pH 2.6

Flow Rate: 1.5 mL/min, 1.0 mL/min

Temperature: 35 °C

Detector: UV, 270 nm

Sample: 2 µL, cough formula



1. Maleic acid
2. Pseudo-ephedrine
3. Acetaminophen
4. Chlorpheniramine
5. Dextromethorphan

Guaifenesin: USP analysis of guaifenesin

Mobile Phase: 40% Methanol:60% Water:1.5% Glacial Acetic Acid

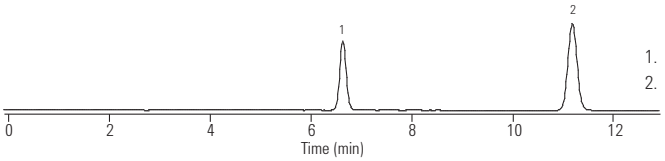
Flow Rate: 1.0 mL/min

Temperature: 25 °C

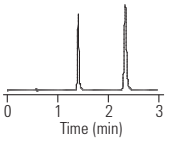
Sample: Guaifenesin
A: 8 µL
B: 2 mL

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

Peak	TR	N	Rs
1	6.63	12,737	0
2	11.19	18,552	15.8



1. Guaifenesin: 0.04 mg/mL
2. Benzoic Acid: 0.10 mg/mL



Column: **Eclipse XDB-C18**
922975-902
4.6 x 50 mm, 1.8 µm

Peak	TR	N	Rs
1	1.4	11,421	0
2	2.33	12,909	12.3

Minimum Resolution Required = 3.0

Metronidazole: Updating USP methods

Column A: **ZORBAX C8**
883952-706
4.6 x 150 mm, 5 µm

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

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

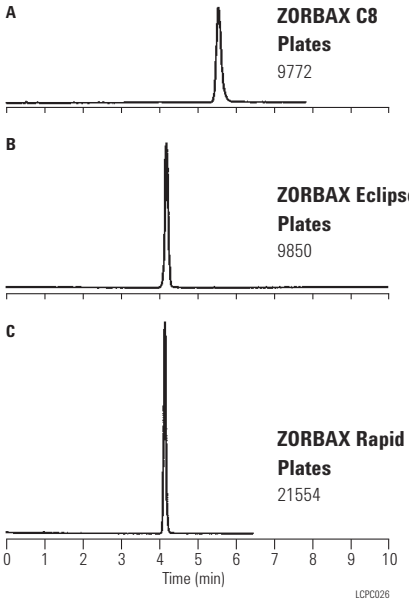
Mobile Phase: 80/20, Water/Methanol

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Metronidazole



A **ZORBAX C8**

Plates	USP TF	Particle Size
9772	1.30	5 µm

B **ZORBAX Eclipse XDB-C8**

Plates	USP TF	Particle Size
9850	0.98	5 µm

C **ZORBAX Rapid Resolution Eclipse XDB-C8**

Plates	USP TF	Particle Size
21554	1.13	3.5 µm

Morphine and metabolites: Extracted blood plasma sample separation

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

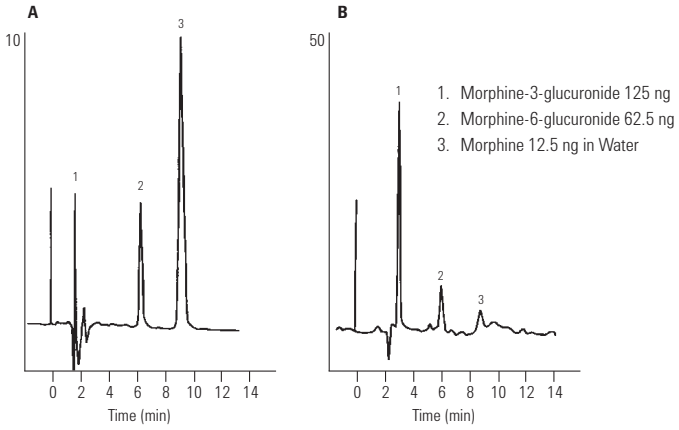
Mobile Phase: 97/3 70 mM KH_2PO_4 + 1 mM EDTA/ACN, pH 4.5

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: A: Electrochemical, 720 mV
B: Fluorescence, Ex = 285 nm, Em = 352 nm

Sample: 50 µL
Morphine-3-glucuronide 125 ng
Morphine-6-glucuronide 62.5 ng
Morphine 12.5 ng in Water



Courtesy of J. Visser, Center for Pharmacy, Univ. Groningen, The Netherlands.

Opiates (drugs of abuse) by LC/MS

Column: **ZORBAX SB-AQ**
830990-914
2.1 x 150 mm, 3.5 µm

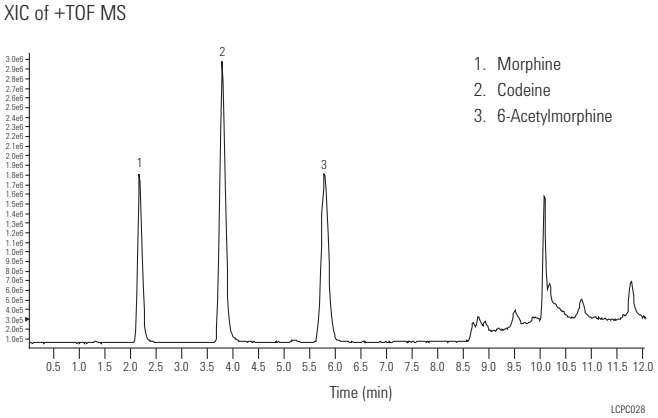
Mobile Phase: A: Acetonitrile with 0.1% formic acid
B: Water with 0.1% formic acid

Flow Rate: 0.25 mL/min

Gradient: 0 min 10% B
5 min 35% B
5.1 min 100% B

MS Conditions: Time of Flight (TOF)
Standard with calibrant delivery system providing constant low flow of ~ 2 µM purine and HP-921 calibrant to dual ESI for continuous auto-calibration

Sample: Opiates



For a comprehensive listing of chromatograms searchable by compound name, visit our online Chromatogram Library at **www.agilent.com/chem/library**

NEW!

Comparing HILIC and RPLC of morphine using Agilent ZORBAX RRHD columns with UHPLC/MS

Column: **Agilent ZORBAX Eclipse Plus C18**
2.1 x 100mm, 5 µm
(Custom column)

Column: **ZORBAX RRHD HILIC Plus**
959758-901
2.1 x 100 mm, 1.8 µm

Mobile Phase: A: 10 mM NH_4HCO_2, pH 3.2
B: CH_3CN/100 mM NH_4HCO_2, pH 3.2 (9:1)
Column A: 10% B isocratic
Column B: 70% B isocratic

Flow Rate: Column A: 0.4 mL/min
Column B: 1 mL/min

Pressure: Column A: 90 bar
Column B: 810 bar

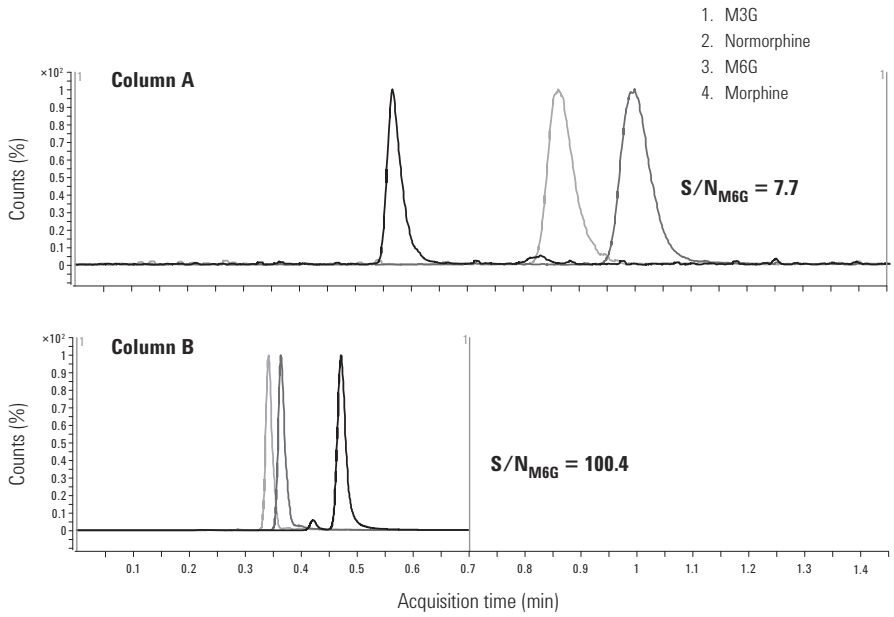
Temperature: 25 °C

Detector: Agilent 1290 Infinity LC with an Agilent 6410A Triple Quadrupole Mass Spectrometer

MS Conditions:
MS Source: Positive ESI, capillary 4000 V, drying gas temperature, flow rate and nebulizer pressure vary with mobile phase flow rate
MS Acquisition: Selected ion mode (SIM), delta EMV 200 V, MS dwell time caries with mobile phase flow rate
Software: Agilent MassHunter versions B.03.01, B.02.00 AND B.03.01 were used for data acquisition, qualitative, and quantitative analyses, respectively

Sample: 2 µL injection of 1 µg/mL each of morphine, normorphine, morphine-3-ß-D-glucuronide: HILIC sample was prepared in CH_3CN; RPLC sample was prepared in H_2O

HILIC mode with UHPLC columns cuts analysis time in half, while improving sensitivity by more than a factor of 10, compared to traditional LC columns in RPLC mode with MS detection.



Neutraceuticals: Hypericin separation in St. John's Wort

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

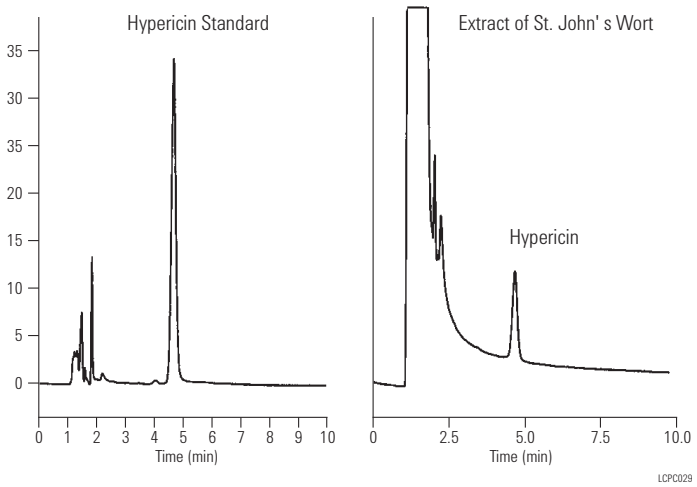
Mobile Phase: 23% 25 mM Na_2HPO_4 ,
Dibasic (pH 7.0 with H_3PO_4): 77% MeOH

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample: Neutraceuticals



Pharmaceuticals: Rapid, high sensitivity LC and LC/MS of 11 drugs

Column: **Eclipse XDB-C18**
925700-902
2.1 x 50 mm, 1.8 µm

Mobile Phase: A: 10 mM NH_4 Formate (pH = 3.6)
B: ACN with 10 mM NH_4 Formate

Flow Rate: 0.6 mL/min

Gradient: 5% B to 70% B in 7.5 min, to 95% B in 8.5 min

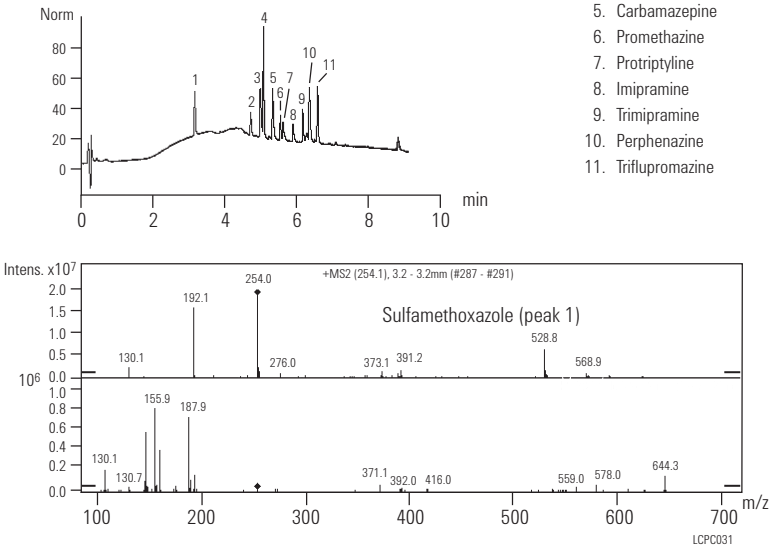
Temperature: 65 °C

Detector: UV, 230 nm and MSD Trap SL

MS Conditions:

Pos. Dry Gas:	345 °C
Neb.:	45 psi
HV Cap:	3500 V
Range:	100-700
Average:	5 Spectra
ICC:	30000
Charge Con:	On
Smart Par. Settings:	Tar Mas: 250 m/z
Comp. Stab.:	100%
Trap Drive:	100%
Frag. Options:	Smart Frag: On
Frag. Width:	10 m/z

1. Sulfamethoxazole
2. Tripelennamine
3. Prednisolone
4. Diphenhydramine
5. Carbamazepine
6. Promethazine
7. Protriptyline
8. Imipramine
9. Trimipramine
10. Perphenazine
11. Triflupromazine



Hormones/steroids

Column: **ZORBAX RRHT SB-C18**
823975-902
4.6 x 30 mm, 1.8 µm

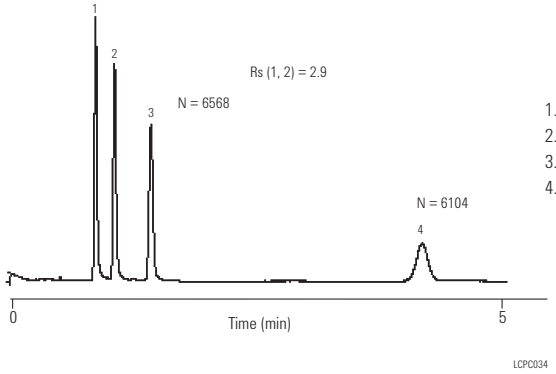
Mobile Phase: 50% 20 mM NaH_2PO_4, pH 2.8: 50% ACN

Flow Rate: 1.0 mL/min

Temperature: RT

Detector: UV, 230 nm

Sample: Hormones/steroids



1. Estradiol
2. Ethinylestradiol
3. Dienestrol
4. Norethindrone

Steroids: Separation

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

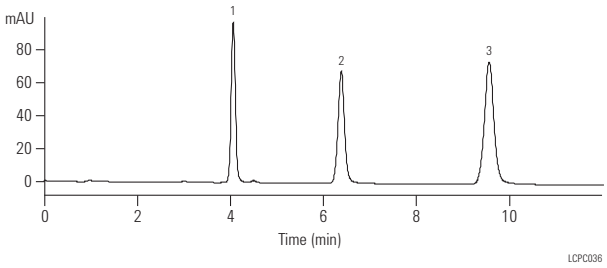
Mobile Phase: 40:60 ACN:Water

Flow Rate: 1.0 mL/min

Temperature: 25 °C

Detector: UV, 205 nm

Sample: 1. Norethindrone 0.514 mg/mL
2. Progesterone 0.407 mg/mL
3. Mestranol 0.057 mg/mL



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Steroids

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

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

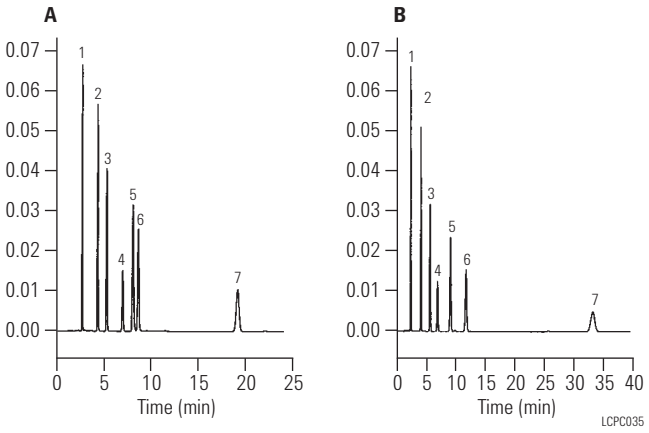
Mobile Phase: H_2O:ACN, 60:40

Flow Rate: 1.0 mL/min

Temperature: 35 °C

Detector: UV, 254 nm

Sample:
1. Prednisolone
2. Corticosterone
3. 11 -hydroxyprogesterone
4. Cortisone acetate
5. Deoxycorticosterone
6. 17 hydroxyprogesterone
7. Progesterone



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Triamcinolone – USP analysis of triamcinolone

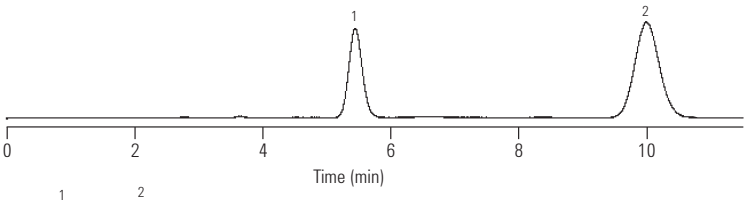
Column: **Eclipse XDB-C18**
923975-902
4.6 x 30 mm, 1.8 µm

Mobile Phase: 47% Methanol:53% Water

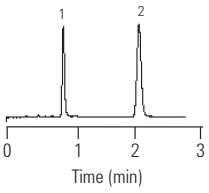
Flow Rate: 1.5 mL/min

Temperature: 25 °C

Sample: Triamcinolone, 1 µL



Peak	TR	N	Rs
1	5.45	3199	0
2	9.99	3212	8.1



1. Triamcinolone: 0.2 mg/mL
2. Hydrocortisone: 0.3 mg/mL

Minimum Resolution Required = 3.0

Peak	TR	N	Rs
1	0.89	3256	0
2	2.07	4851	11.8

LCPC038

Separation of highly basic antidepressants above their pKa in free base form (pKa 9.5-9.7)

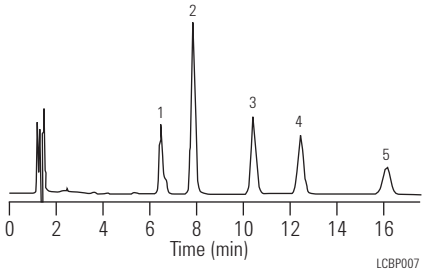
Column: **ZORBAX Extend-C18**
773450-902
4.6 x 150 mm, 5 µm

Mobile Phase: 75% Methanol / 25% 50 mM Pyrrolidine Buffer, pH 11.5

Flow Rate: 0.5 mL/min

Temperature: 40 °C

Detector: UV, 215 nm



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

LCBP007

Basic drugs can often be separated in their charged form at low pH with StableBond or at mid-range pH with Eclipse XDB or Bonus -RP columns. With Extend-C18, you can separate at high pH to improve solubility, improve retention, or obtain different selectivity.

Antidepressants, tricyclic: Comparative separation

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

Column B: **Brand A Polar-linked C8**

Column C: **Brand B Polar-linked C18**

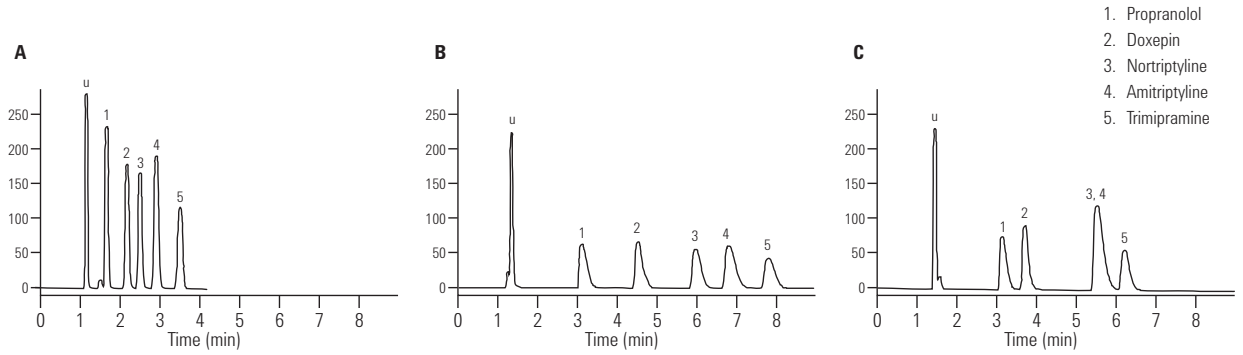
Mobile Phase: ACN: 20 mM Na Citrate, pH 6 (60:40)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Tricyclic antidepressants (u= uracil)



LCBP011

Tricyclic antidepressants

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

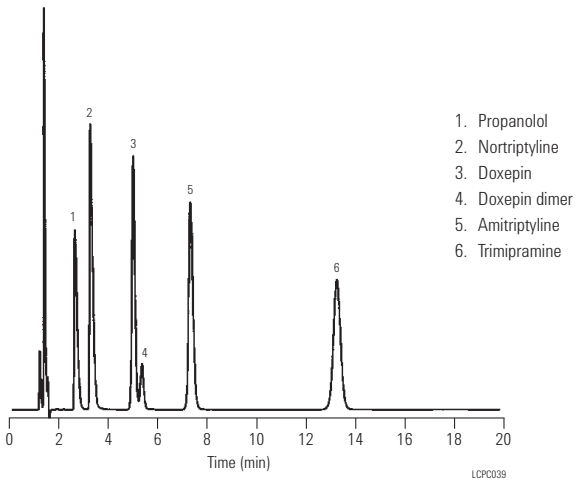
Mobile Phase: 38/62 THF/25 mM Potassium Phosphate, pH7

Flow Rate: 1.0 mL/min

Temperature: 23 °C

Detector: UV, 254 nm

Sample: 10 µL, Antidepressant mix, 10 µg/mL



LCPC039

Tricyclic antidepressants and metabolites: Effect of pore size

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

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

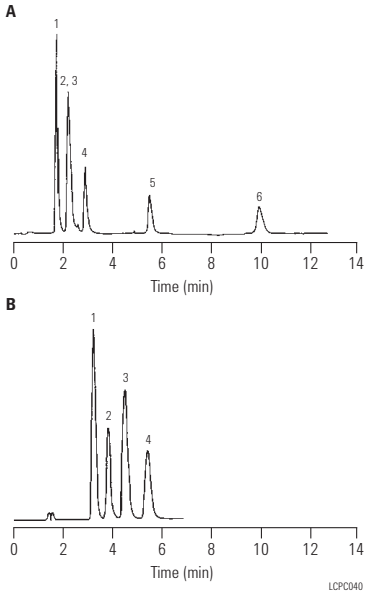
Mobile Phase: 40/60, 25 mM Phosphate Buffer, 10 mM Triethylamine, pH 6.2/ACN

Flow Rate: 1.2 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 10 µL, Antidepressant mix, 10 µg/mL



1. trans- 10-OH - Nortriptyline
2. trans- 10-OH - Amitriptyline
3. cis- 10-OH - Nortriptyline
4. cis- 10-OH - Amitriptyline
5. Nortriptyline
6. Amitriptyline

Ulcer treatment drugs at intermediate pH

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

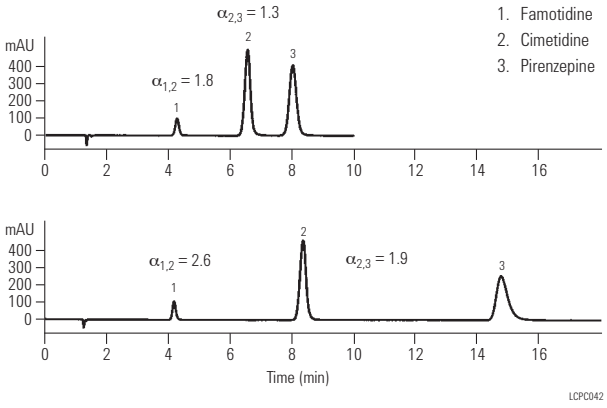
Mobile Phase: Na citrate, 20 mM, pH 6.1: MeOH, (80:20)

Flow Rate: 1.0 mL/min

Temperature: Ambient

Detector: UV, 220 nm

Sample: Ulcer treatment drugs



1. Famotidine
2. Cimetidine
3. Pirenzepine

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Urine, LSD analysis by LC/MS

Column: **Eclipse XDB-C8**
960967-906
2.1 x 50 mm, 5 µm

Mobile Phase: 15 : 85, ACN : 10 mM Ammonium Formate, pH 3.7

Flow Rate: 0.3 mL/min

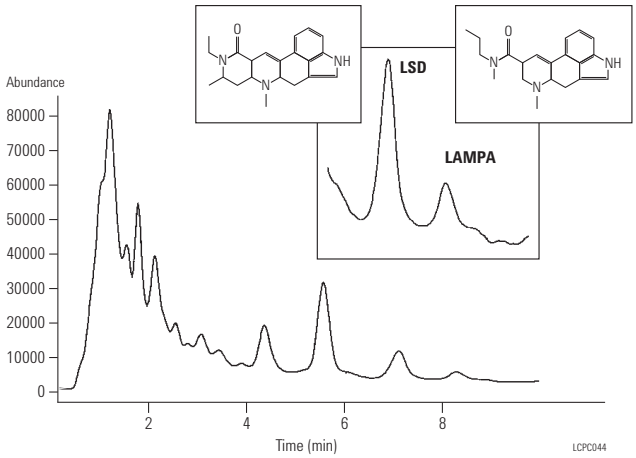
Temperature: 30 °C

Detector: MS

MS Conditions: SIM mode, Ions: 324.2, 223.1, 208.1
Fragmentor (dynamically ramped) 100V at 324.2, 148V at 223.1, 170V at 208.1

Sample: LSD

Hughes, J.M., C.A. Miller and S.M. Fischer, "Development of a Method for the Forensic Analysis of LSD in Urine", presented at the ASMS, Palm Springs, June 1997.



USP method:
Glyburide and internal standard, progesterone

Column: **Eclipse XDB-C8**
990967-906
4.6 x 250 mm, 5 µm

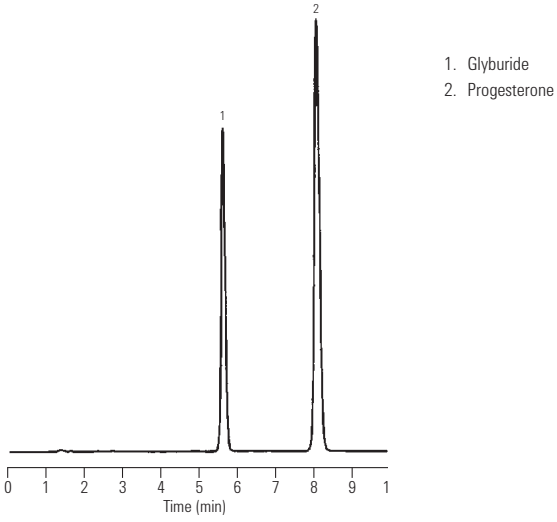
Mobile Phase: 45/55, 50 mM Ammonium Phosphate/ACN, Final pH 5.35

Flow Rate: 1.5 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: 5 µL, 10 ug/mL each of standard



1. Glyburide
2. Progesterone

Dexamethasone, USP method: Rapid analysis

Column A: **ZORBAX SB-C8**
880975-906
4.6 x 250 mm, 5 µm

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

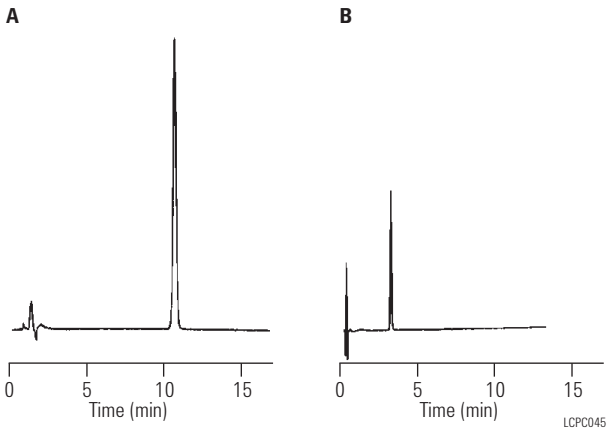
Mobile Phase: A = Water, B = ACN; Isocratic 30% B

Flow Rate: 2.0 mL/min

Temperature: Ambient

Detector: UV, 254 nm

Sample: Dexamethasone
10 µL and 5 µL, 10 ug/mL



USP analysis of tetracyclines

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

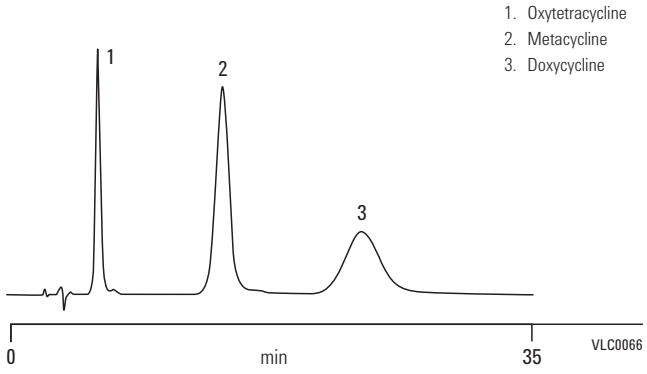
Sample: 20 mg tetracycline in 25 mL 0.01M HCl

Mobile Phase: 60 g 2-Methyl-2-propanol + 200 mL UHP water + 400 mL 0.2 M K_2HPO_4 at pH 8 + 50 mL 10 g/L tetrabutylammonium hydrogen sulphate at pH 8 + 10 mL 40 g/L sodium edetate at pH 8, made up to 1000 mL with water (adjust pH with dilute NaOH)

Flow Rate: 1.0 mL/min

Temperature: 60 °C

Detector: UV, 254 nm



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Warfarin: USP chromatographic purity method using Eclipse XDB-CN

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

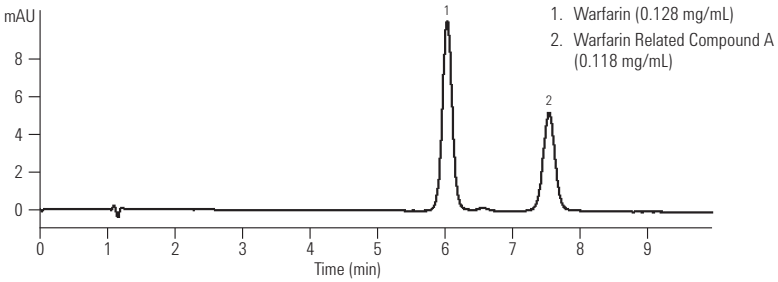
Mobile Phase: 32:68:1 Acetonitrile:Water:Glacial Acetic Acid

Flow Rate: 1.5 mL/min

Temperature: 25 °C

Detector: UV, 260 nm

Sample: Warfarin, 2 µL



Ten cardiac drugs on Rapid Resolution HT SB-C18

Column: **SB-C18**
829975-902
4.6 x 150 mm, 1.8 µm

Mobile Phase: A: 0.1% TFA, 5% ACN
B: 0.08% TFA, 95% ACN

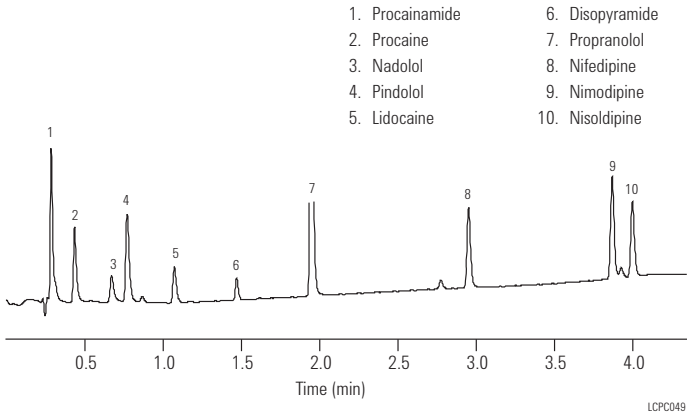
Flow Rate: 2 mL/min

Gradient: 0.0 min 12.5% B
10.5 min 60% B
12.0 min 60% B

Temperature: 70 °C

Detector: UV, 230 nm

Sample: Cardiac drugs



Sulfonamides – Fast analysis with RRHT columns

Column: **SB-C18**
824700-902
2.1 x 30 mm, 1.8 µm

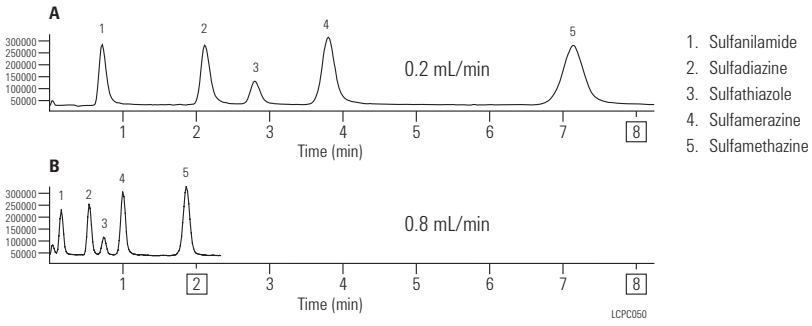
Mobile Phase: A: 90% 0.1% formic acid
B: 10% 0.1% formic acid in MeOH

Flow Rate: A: 0.2 mL/min
B: 0.8 mL/min

Temperature: 35 °C

Detector: TIC, Single Quad

Sample: Sulfonamides



Sulfa drugs

Column: **Pursuit XRs Ultra C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

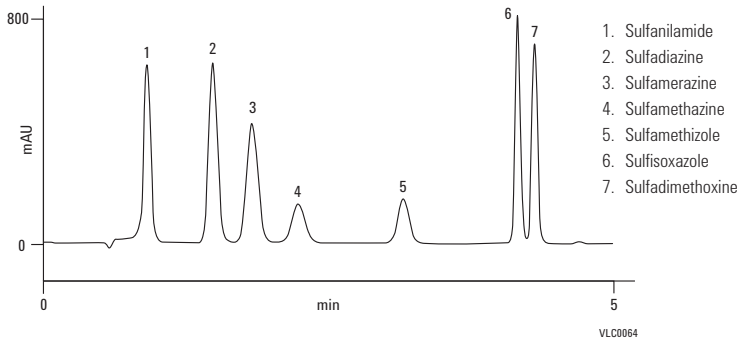
Mobile Phase: A: Water+0.1% TFA
B: MeCN+0.1% TFA

Gradient: 10% B for 10 min,
ramp to 45% B in 1 min and hold for 1 min,
return to 10% B in 1 min and hold for 1 min

Flow Rate: 0.65 mL/min

Temperature: Ambient

Detector: UV, 254 nm



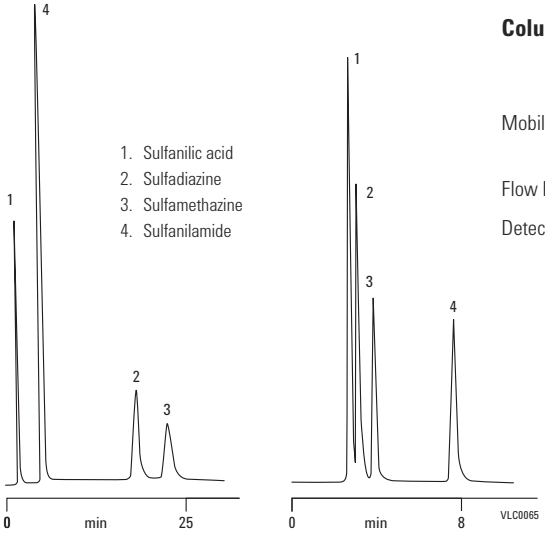
Sulfa drugs

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

Mobile Phase: Potassium sulfate:
ACN 7:1, pH 2.2

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm



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

Mobile Phase: Disodium tetraborate: ACN 6:1,
pH 9.3

Flow Rate: 1.0 mL/min

Detector: UV, 254 nm

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Fast analysis of Pindolol

Column A: **ZORBAX SB-CN**
863953-905
4.6 x 150 mm, 3.5 µm

Column B: **ZORBAX SB-CN**
827975-905
4.6 x 50 mm, 1.8 µm

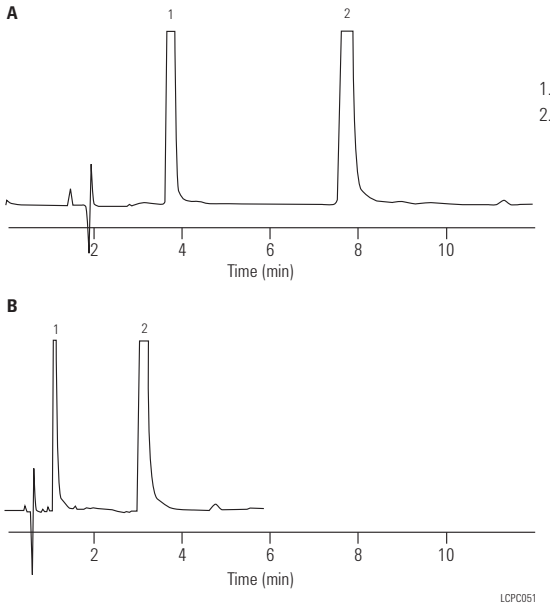
Mobile Phase: A: 70% 50 mM Na Acetate
B: 30% ACN

Flow Rate: 1 mL/min

Temperature: Ambient

Detector: UV, 219 nm

Sample: Pindolol, 2 µL



1. Indole
2. Pindolol

Lamotrigine

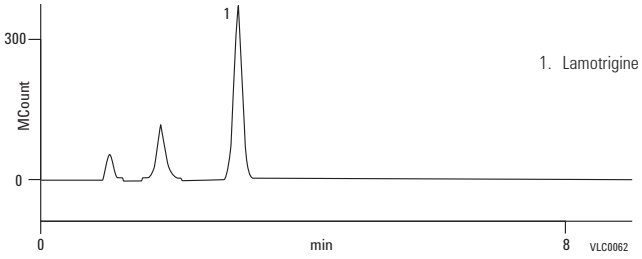
Column: **Pursuit XRs Ultra C8**
A7511100X020
2.0 x 100 mm, 3.0 µm

Mobile Phase: ACN:water, 25:90 for 1 min

Flow Rate: 0.2 mL/min

Injection Volume: 5 µL, 50% MeOH

Detector: MS



1. Lamotrigine

Barbiturates

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

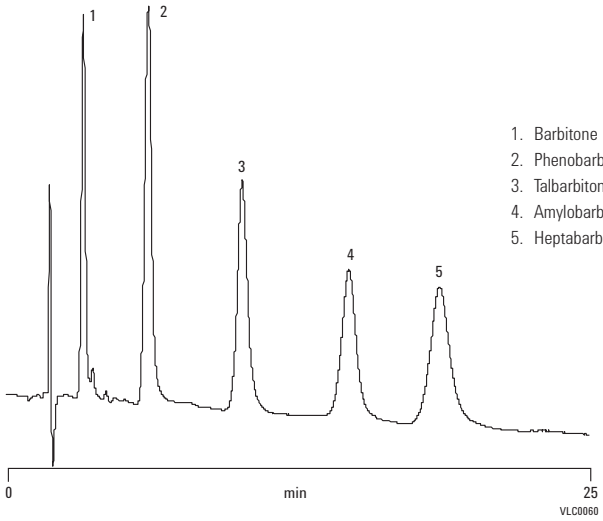
Mobile Phase: Water

Flow Rate: 1.0 mL/min

Temperature: 200 °C

Detector: UV, 220 nm

Courtesy: Smith, RM, Burgess, RJ, Cheinthavorn, O and Stuttard, JR (1999) Superheated water: a new look at chromatographic eleunts for reversed-phase liquid chromatography. LCGC Europe, January 1999, 30-36. Used with permission.



1. Barbitone
2. Phenobarbitone
3. Talbarbitone
4. Amylobarbitone
5. Heptabarbitone

Analysis of ciprofloxacin and ciprofloxacin metabolites

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

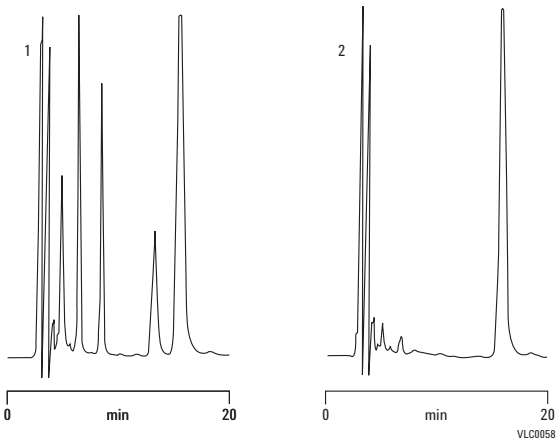
Mobile Phase: 74% 20 mM TCA:22% ACN:4% MeOH adjusted to pH 3

Flow Rate: 1.0 mL/min

Detector: UV, 277 nm

Krol GJ, Noe, AJ and Beerman, D (1986) Liquid chromatographic analysis of ciprofloxacin and ciprofloxacin metabolites in body fluids. Journal of Liquid Chromatography, 9(13), 2897-2919. Reprinted with permission of the publisher (Taylor & Francis Group, www.informaworld.com).

1. Blank urine sample containing known concentrations of internal standard, ciprofloxacin and its metabolites
2. Blank urine sample containing only internal standard



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