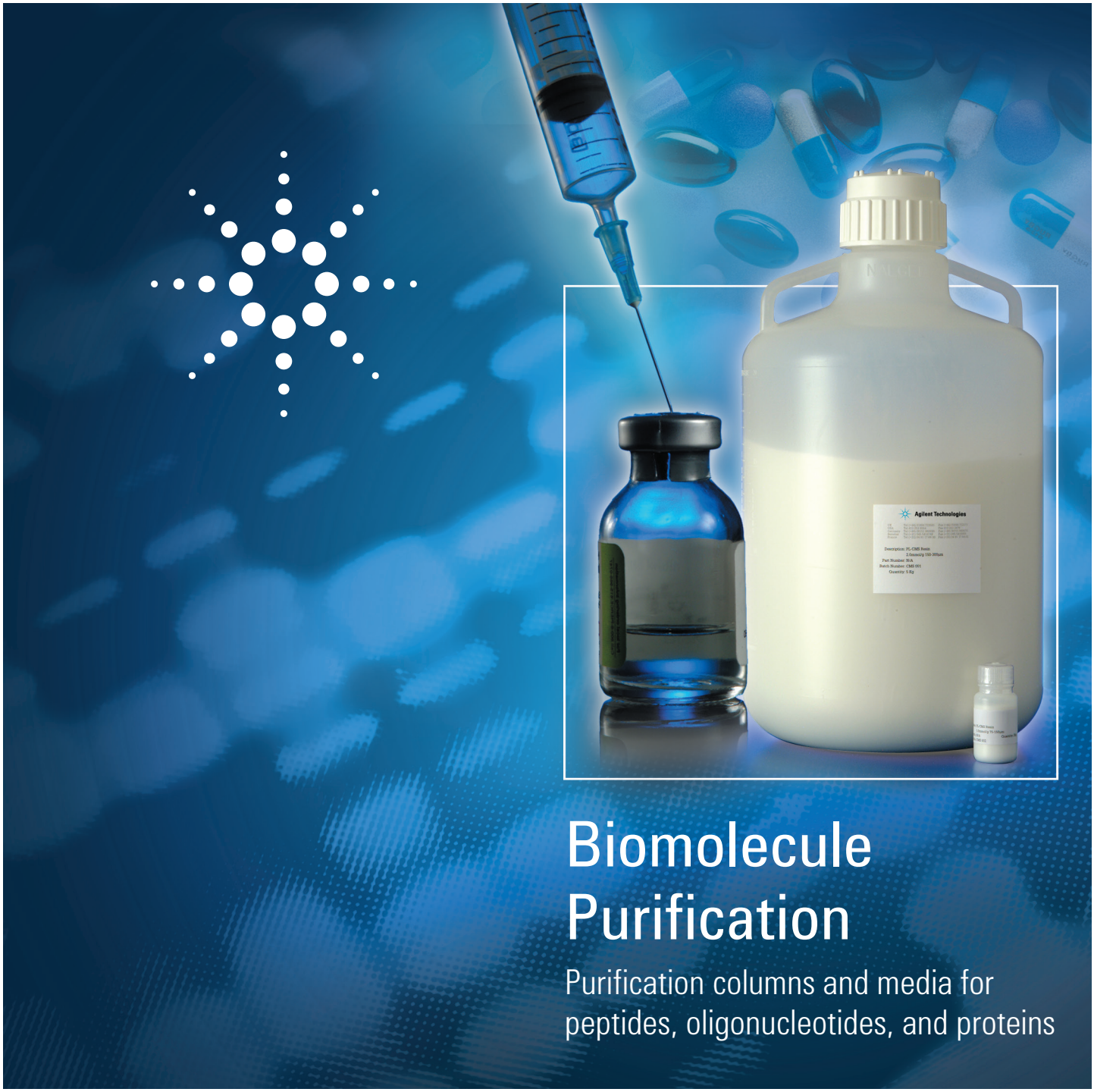


Biomolecule Purification

Purification columns and media for peptides, oligonucleotides, and proteins

The Measure of Confidence

Agilent Technologies

Highest quality, highest performance

Rigid polymerics for high-quality prep-to-process chromatography

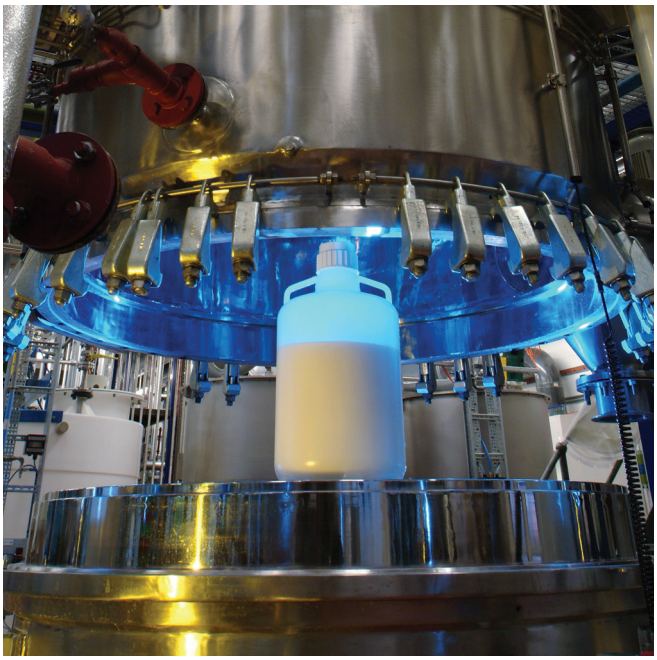
With the acquisition of Varian and the Polymer Laboratories portfolio, Agilent is now a leading manufacturer of specialty polymeric particles for chromatographic, pharmaceutical, and diagnostic applications.

Our high-performance particles are manufactured by controlled polymerization and modification of polystyrene/divinylbenzene (PS/DVB). Our proprietary production processes give complete control over pore and particle size and surface chemistry, ensuring reproducibility from lot to lot. The optimized pore structure provides the chromatographic characteristics required for the purification of a wide range of biomolecules, from small peptides and oligonucleotides through to large proteins and DNA fragments.

- Agilent PLRP-S is a rigid, macroporous media for reversed-phase chromatography.
- Agilent PL-SAX is a strong anion exchanger.
- Agilent PL-SCX is a strong cation exchanger.

Key benefits

- Cleaner media and the absence of particle degradation deliver product free of contamination to increase compound purity.
- Range of optimized pore sizes and structures and chemical stability increase sample loading and throughput.
- Batch-to-batch reproducibility provides consistent chromatography, giving you confidence in your results.
- Column stability and the capacity to withstand aggressive clean-in-place procedures deliver long column lifetimes to improve productivity.



With a manufacturing capacity in excess of 4 metric tons, Agilent is one of the world's largest bulk media suppliers.

A legacy of purification column and media innovation	
1976	PS/DVB particle production begins.
1984	PLRP-S reversed-phase media is introduced.
1986	The manufacture of preparative HPLC media starts. PL-SAX strong anion-exchange media is introduced.
1988	Diagnostic latex particles are introduced.
1992	Solid phase synthesis supports are introduced.
1997	ISO 9001 accreditation.
1999	The manufacture of process-scale HPLC media begins.
2002	Process-scale bulk media is manufactured in a new state-of-the-art production facility.
2005	Polymer Laboratories is acquired by Varian, Inc.
2007	The prep-to-process team with instrument solutions is formed.
2009	SepTech reversed-phase media is introduced.
2010	Varian is acquired by Agilent Technologies, Inc.

The future of prep-to-process chromatography

Parallel performance

In reversed-phase chromatography, it would be expected that the hydrophobicity of alkyl-bonded silica would differ significantly from that of the aromatic groups present on the surface of a PS/DVB matrix. However, the observed capacity factor k′ (calculated from the formula $k' = (t_R - t_0)/t_0$ where t_0 is the retention time for a non-sorbed solute) for four peptides on leading C_{18} and C_4 silica columns and on PLRP-S columns demonstrates similarity of retention but differences in selectivity.

Conditions

Columns	PLRP-S and silica, 4.6 x 250 mm
Eluent A	0.1% TFA in 20% ACN/80% water
Eluent B	0.1% TFA in 50% ACN/50% water
Gradient	0-100% B in 15 min
Flow rate	1.0 mL/min
Detector	UV, 220 nm

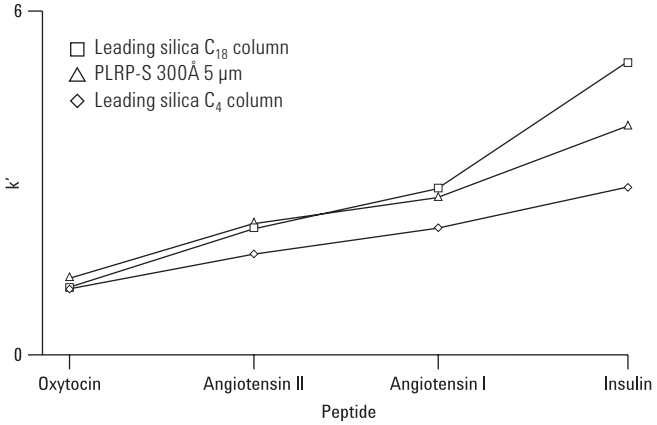


Figure 1. Comparison of retention characteristics: PLRP-S and silica-based packing.

However, column/media lifetimes cannot be similarly compared; the long-term chemical and physical stability of Agilent's polymeric media ensures reproducible resolution with greatly extended column lifetimes, when aggressive cleaning-in-place (CIP) regimes are needed or when separations are performed at extremes of pH or high temperature.

Solvent compatibility

Exceptionally low swelling is achieved via the high crosslinked density of the polymer structure, ensuring complete gradient compatibility with the widest range of organic modifiers. Using PLRP-S, it is not necessary to dedicate columns to particular organic modifiers as transfer between modifiers can be rapidly achieved.

Typical mobile phase modifiers		
Acetonitrile	Ethanol	n-Propanol
Acetone	Isopropanol	Tetrahydrofuran
Dimethylsulfoxide	Methanol	

Gradient stability

With PLRP-S media, operating pressure and changing pressure through an aqueous/organic gradient are comparable to that of silica-based materials, and lower than observed with a lower crosslinked polymeric material. No change in swelling in the PLRP-S media is detected as the organic content of the eluent changes.

Conditions

Columns	PLRP-S and silica, 4.6 x 250 mm
Eluent A	0.1% TFA in 20% ACN/80% water
Eluent B	0.1% TFA in 50% ACN/50% water
Gradient	0-100% B in 15 min
Flow rate	1.0 mL/min
Detector	UV, 220 nm

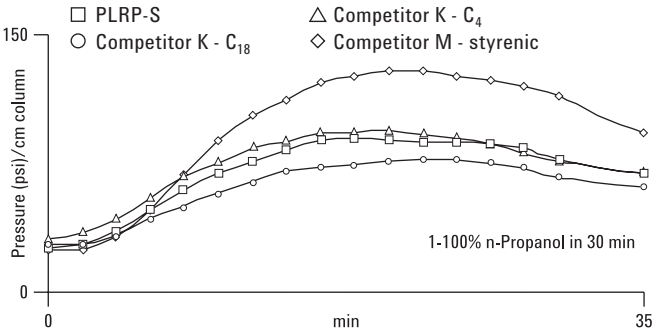


Figure 2. Pressure through aqueous/organic gradient, showing the pressure throughout the gradients seen with the PLRP-S materials comparible to silica-based media.

Chemical and pH stability

Enhanced selectivity for improved loading

PLRP-S, PL-SAX, and PL-SCX media are stable over the full pH range. The apparent hydrophobicity and net ionic charge of the target molecule and/or impurities can be manipulated by controlling pH to enhance selectivity.

Traditionally, peptide separations were performed at acidic pH, within the limited pH stability range of silica-based materials. The absence of silanols in PLRP-S media permits the use of low levels of trifluoroacetic acid (TFA) for reversed-phase separations. Alternatively, separations can be done under neutral and basic pH conditions, improving purification resolution and loading.

Conditions

Column	PLRP-S 100Å 10-15 µm, 4.6 x 250 mm
Eluent A	Water plus modifier as detailed on each chromatogram above
Eluent B	ACN plus modifier as detailed on each chromatogram above
Flow rate	1.0 mL/min
Detector	UV, 220 nm

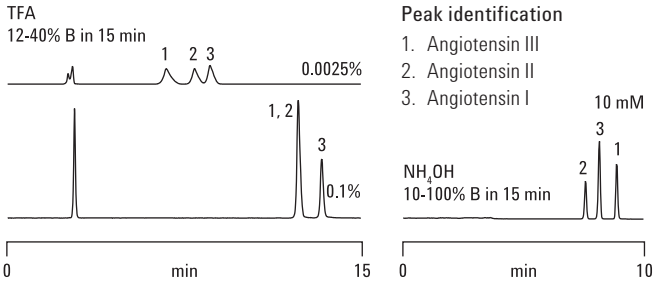


Figure 3. Effect of pH on peptide selectivity.

Cleaning in place

Agilent's media is chemically robust and can withstand extremely aggressive sanitizing/cleaning protocols. Media can be cleaned in a packed column under CIP, or in bulk, using a range of solubilizing agents, such as NaOH, to ensure unsurpassed column and media lifetimes.

Conditions

Column	PLRP-S 300Å 10-15 µm, 4.6 x 250 mm
Eluent A	0.1% TFA in water
Eluent B	0.1% TFA in ACN
Gradient	20-50% B in 15 min
Flow rate	1.0 mL/min
Detector	UV, 220 nm

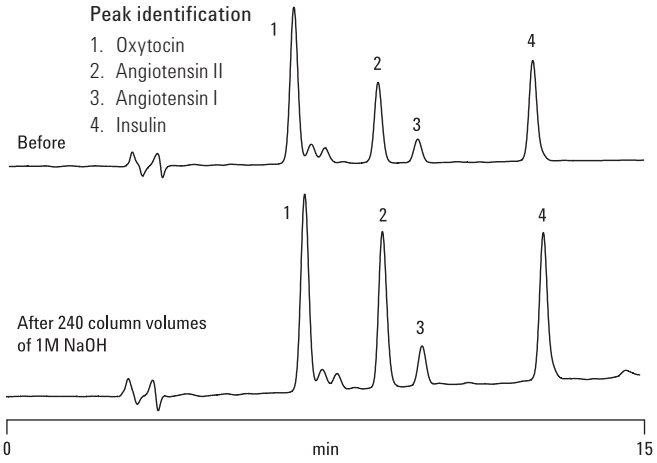


Figure 4. 1 M sodium hydroxide stability.

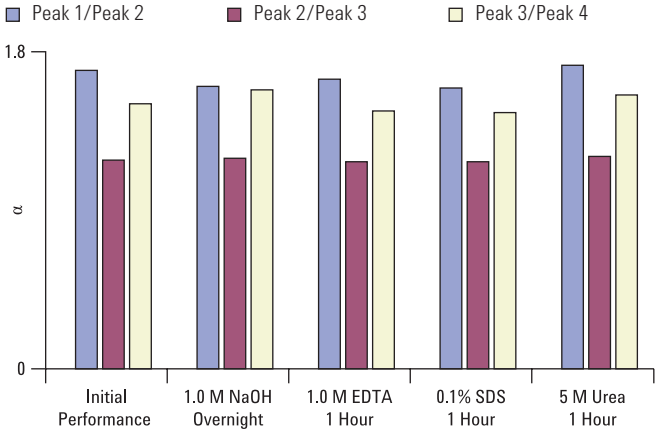


Figure 5. Peptide selectivity after CIP procedures.

Physical and thermal stability

Physical stability

Our particles are physically robust and stable up to 6000 psi. They can be packed in high-performance, high-pressure column hardware and also in dynamic axial compression (DAC) systems. There is no compression of the particle when operated under HPLC conditions of pressure and flow rate.

For maximum process economics and throughput, the linear velocities used for the purification and the operating pressure must be compatible with the equipment being used. We therefore produce a range of particle sizes to cover the range of system operating pressures.

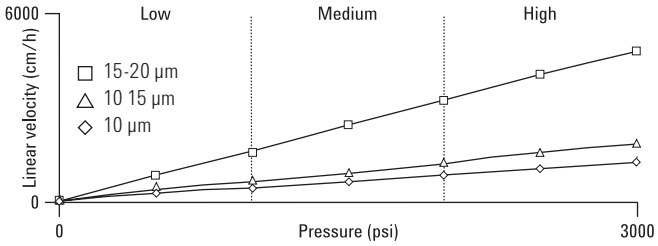


Figure 6. Pressure vs linear velocity.

Thermal stability

Our media is thermally stable for separations that require elevated temperatures, such as oligonucleotide separations, which are routinely run at 60 °C.

Conditions

Column	PL-SAX 1000Å 8 µm, 4.6 x 50 mm
Eluent A	93% 0.1 M TEAA, pH 8.5/7% ACN
Eluent B	93% 0.1 M TEAA, 1 M ammonium chloride, pH 8.5/7% ACN
Gradient	0-40% B in 10 min, 40-70% B in 14 min, 70-100% B in 25 min
Flow Rate	1.5 mL/min
Temperature	60 °C

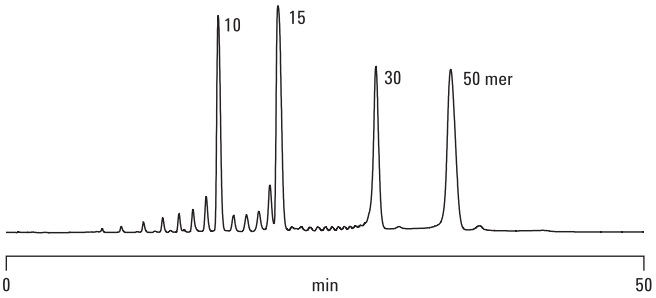


Figure 7. Separation of a poly T-standard at 60 °C.

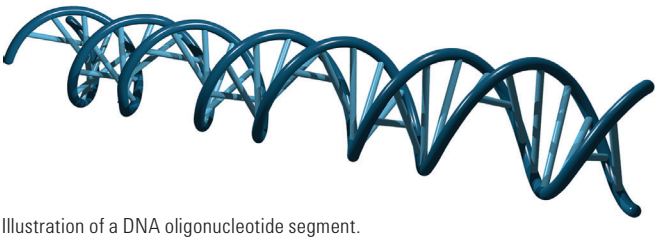


Illustration of a DNA oligonucleotide segment.

Optimized column lifetime and pore structure

Unsurpassed column lifetimes

The rigidity of polymeric particles prevents compaction and ensures packed-bed stability under biotherapeutic purification conditions of high linear velocity. You get faster run times for higher throughput and more cycles between repacking, improving process economics.

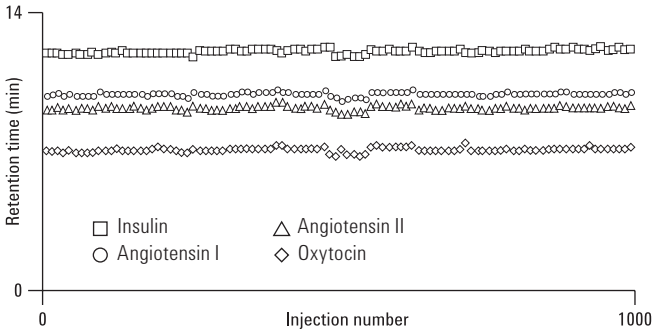


Figure 8. Retention times, resolution, and column efficiency remain unchanged after 1,000 injections.

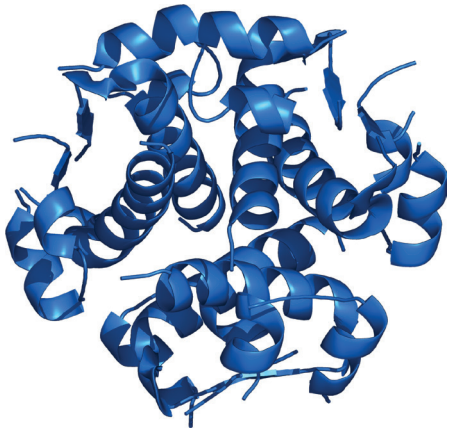


Illustration of an insulin structure.

Optimized pore structure

To maximize throughput, it is essential that media has maximum loading capacity. Loading is influenced by the pore size, pore-size distribution, and pore volume as these parameters determine the available surface area. The size of the molecule to be purified determines the minimum pore diameters of the media.

Table 1. Capacity mg/mL CV.

Column	Pore size (Å)	Insulin	Lysozyme	BSA	Oligos
PLRP-S	100	90	55	5	72
	300	60	45	25	54
PL-SAX	1000	-	-	80	-
	4000	-	-	35	-
PL-SCX	100	-	65	-	-
	4000	-	20	-	-

Optimized pore structure ensures excellent mass transfer characteristics. Peptide feedstock can be loaded at high linear velocity with minimal reduction in dynamic capacity.

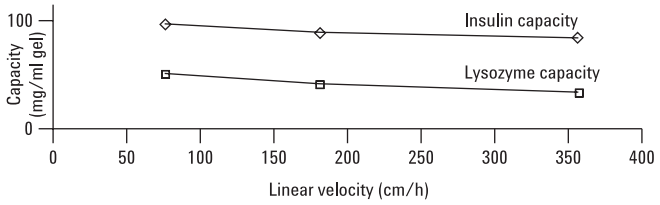


Figure 9. Capacity dependency on linear velocity, PLRP-S 100Å

No leachables and improved economics

Stable polymeric particles deliver a cleaner bioproduct

Our stringent production process removes manufacturing byproducts, producing the cleanest media possible. Our media does not suffer from base particle degradation or bonded-phase stripping, unlike conventional silica reversed-phase material. The result is no contamination of your product with silica and/or alkyl ligands and guaranteed run-to-run reproducibility.

Conditions

Column	Silica C_{18}, 4.6 x 250 mm
Eluent A	75% 0.01 M phosphate pH 2.5/25% ACN
Eluent B	25% 0.01 M phosphate pH 2.5/75% ACN
Gradient	5-30% B in 30 min
Flow rate	1.0 mL/min
Detector	UV, 220 nm

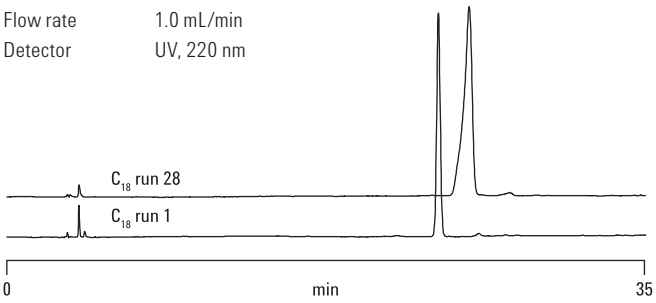


Figure 10. Silica degradation with a basic peptide solution.

As seen in Figure 10, a new silica C_{18} column assessed the purity of the fractions collected from a synthetic peptide purification run at pH 9. After only 28 injections into a buffered low pH system, the column retention characteristics had changed, and efficiency was lost. The PLRP-S column suffered no such deterioration.

Vastly improved process economics

Unit-volume costs for Agilent polymeric media are comparable with the leading process-reversed-phase silica or 'softer' polymerics. In addition, PLRP-S, PL-SAX, and PL-SCX media deliver benefits in throughput, economy, and confidence.

Features	Benefits
Cleaner media means no leachables.	No product contamination, so no time wasted in further cleaning steps.
Chemical stability means no particle degradation or bonded phase stripping.	
Range of pore sizes for maximum loading.	Increased throughput.
Optimized pore structure permits high feed rates.	
Mechanical stability allows use of high linear velocities.	
Chemical stability enables pH to be used as a variable to enhance selectivity and improve loading.	
Batch-to-batch reproducibility gives column-to-column consistency.	Consistent chromatography for confidence in results.
Chemical stability gives reproducibility throughout the column lifetime.	
Rigid particles give packed column stability.	Significantly longer column lifetimes for economic operation.
Chemical stability allows aggressive CIP for 'dirty' feedstocks and column regeneration.	
No production downtime due to column depacking/repacking/ validation.	

Typically, when high pH or temperatures are used to improve selectivity or aggressive CIP is needed, up to four silica columns may be needed to produce the same amount of purified product as one PLRP-S column.

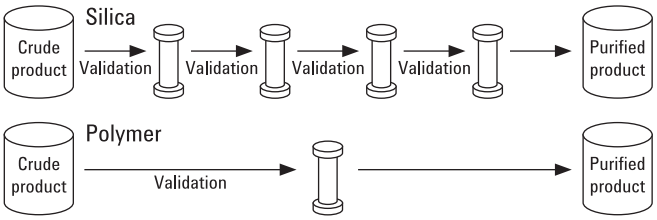


Figure 11. Improved process economics reduce production cost.

Synthetic peptides

Reversed-phase

- Exceptional selectivity.
- Ease of scale-up, µg to multi kg.
- High efficiency at every scale, analytical through process.
- Chromatography across the entire pH range.

Peptide analysis and purification

By using an eluent pH at which the impurity, A, elutes before the product, B, self-displacement is used to increase the throughput of the purification.

Conditions

Column	Prochrom LC110, 25 mm id, packed with 40 g of PLRP-S 300Å 8 µm
Prep load	6 g on-column

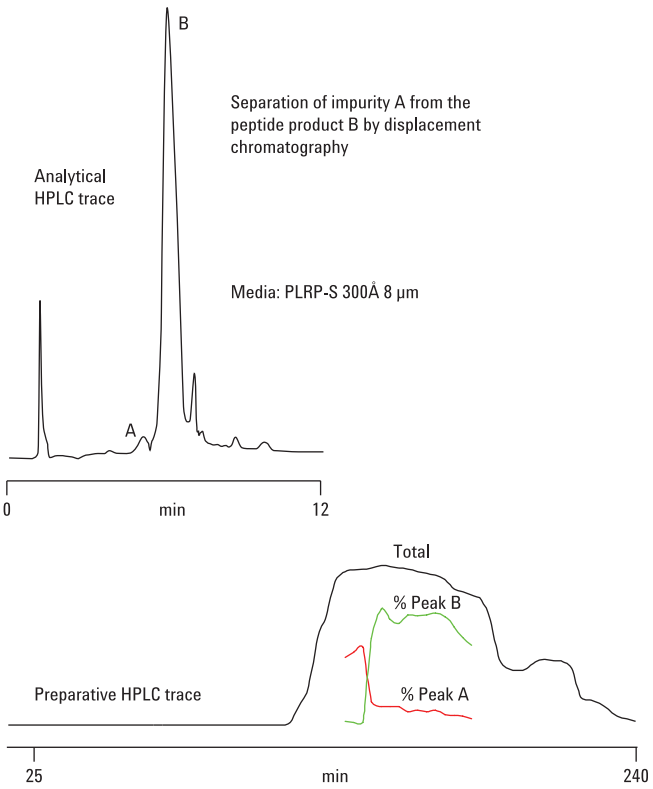


Figure 12. Purification of a 25 amino acid peptide using PLRP-S 300Å in the self-displacement mode.

High capacity/high speed

To maximize throughput, the chromatographic media must be able to operate at high linear velocity and with high sample loading. The optimized pore size and high mechanical stability of PLRP-S media are ideally suited to deliver high throughput and therefore improved process economics.

Purification of a synthetic peptide

Using PLRP-S 100Å 10 µm media to maximize productivity, synthetic peptides can be subjected to a purification regime under overload conditions.

Conditions

Sample	Phe-ile-val tripeptide
Column	PLRP-S 100Å 10 µm
Eluent A	0.1% TFA in 95% water
Eluent B	0.1% TFA in 50% ACN
Gradient	0-100% B in 20 min
Linear velocity	360 cm/h
Detector	UV, 220 nm

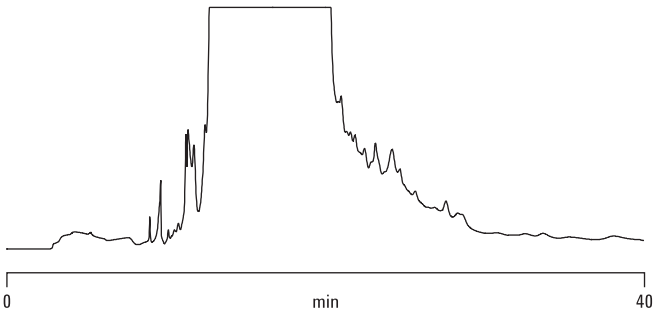


Figure 13. Purity was 95% and yield was 86% in a single purification run.

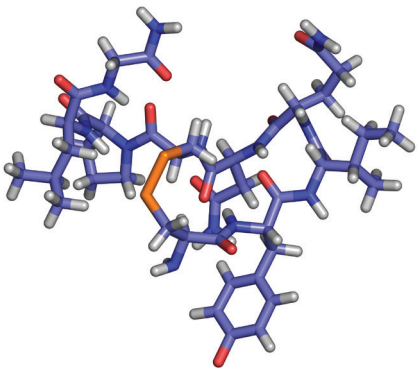


Illustration of an oxytocin structure.

Recombinant peptides/proteins

Reversed-phase

- Optimized 300Å and 1000Å pore sizes.
- High resolution for polishing.
- Tolerant of robust CIP procedures.
- Depyrogenation with 1 M NaOH.

Lysates of escherichia coli

Lysates of E. coli can be injected directly onto a PLRP-S column after filtration to remove cell debris/insoluble material. The elution profiles for a standard lysis buffer and the commercial BugBuster lysis buffer are shown below.

Conditions	
Column	PLRP-S 300Å 10 µm
Eluent A	0.1% TFA in 95% water
Eluent B	0.1% TFA in 80% ACN
Gradient	0-100% B in 20 min, 100% B for 5 min
Linear velocity	360 cm/h
Detector	UV, 220 nm

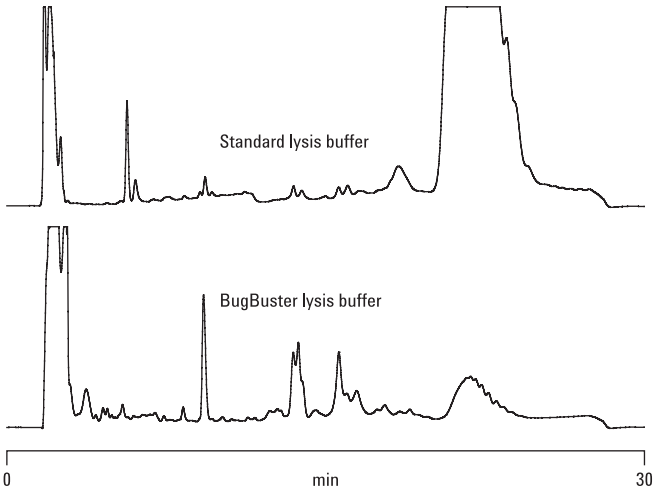


Figure 14. BugBuster lysis buffer illustrates the complexity of the sample matrix.

Clean-in-place regimes

The E. coli lysate is an extremely complex matrix containing a wide range of cellular components. To assess the robustness of PLRP-S media, 200 µL aliquots of the filtered crude lysate were injected.

After 95 injections of the crude lysate, column performance deteriorated. Pressure increased, plate count declined, and the separation of a standard peptide mixture worsened. A strict CIP regime of 1 M NaOH/ACN and THF/ACN was then used. As the separation of four synthetic peptides illustrates, after CIP, the column pressure came down, the plate count was restored, and the peptide separation matched that of the 'new' column.

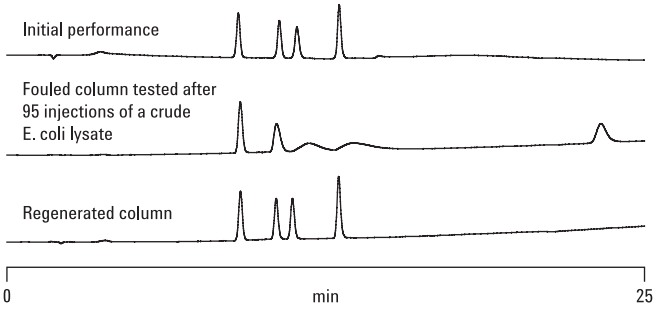


Figure 15. Column evaluation by separation of synthetic peptides, showing complete regeneration of a PLRP-S column after a CIP regime.

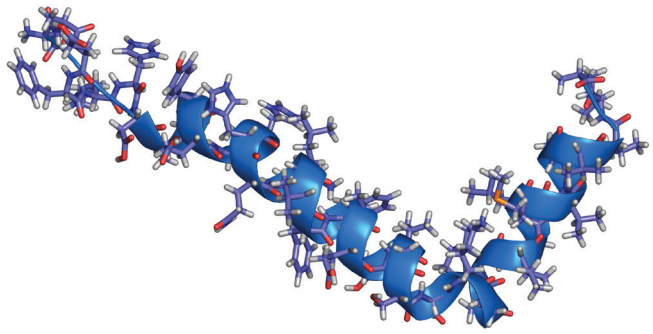


Illustration of a peptide structure implicated in Alzheimer's disease.

Synthetic oligonucleotides

Anion exchange

- High-performance chromatography at high pH.
- Excellent temperature stability.
- Large pore size optimized for large biomolecules.
- High resolution.

Use of high pH improves chromatography where an oligonucleotide is prone to self-association or aggregation.

Thiolated oligonucleotide

Using PL-SAX material, a high pH eluent can be used to separate a fully thiolated oligonucleotide from an impurity where thiolation is incomplete.

Conditions

Sample	Thiolated oligonucleotide
Column	PL-SAX 1000Å 10 µm
Eluent A	1 M NaOH
Eluent B	1 M NaOH, 2 M NaCl
Gradient	75-100% B in 25 min, held at 100% B for 15 min
Linear velocity	360 cm/h
Detector	UV, 260 nm

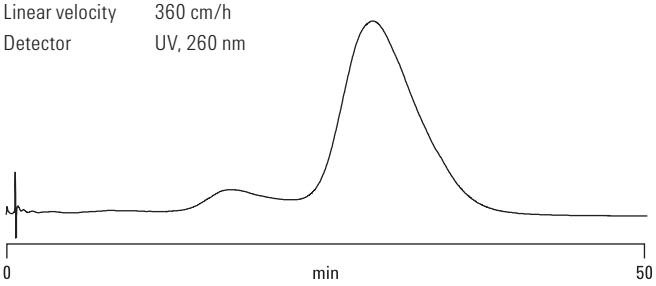


Figure 16. The strong anion-exchange functionality on a chemically inert polymeric matrix gives charge differentiation even in 1 M NaOH.

Oligonucleotide purification

For many applications, the full length oligo(n) is purified from the failure sequences, including n-1.

Conditions

Column	PLRP-S 100Å, 50 x 4.6 mm ID
Sample	100 mmol purification

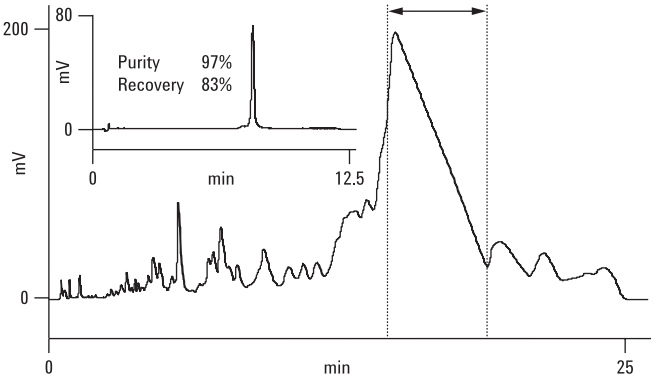


Figure 17. Purification of a 25-mer trityl-off oligonucleotide and analytical quantitation of the fraction using PLRP-S 100Å (inset).

Reversed-phase

Trityl-on/trityl-off

The optimized pore sizes of the reversed-phase PLRP-S media (100Å and 300Å) make it ideal for oligonucleotide purification. For separation of trityl-on/trityl-off oligonucleotides, the high available surface area and homogeneous clean surface of the PLRP-S media give maximum load and recovery.

Ion-pair chromatography

For dynamic ion-pair chromatography, the small particle size PLRP-S provides high-resolution separations.

Table 2. 20-mer oligonucleotide capacity on PLRP-S 100Å media with tetra-n-butylammonium bromide (TBAB) ion pairing agent.

Temperature	Loading (mg/mL)
60 °C	72
Ambient	63

Ordering information

Prep columns and media

Agilent PLRP-S, PL-SAX, and PL-SCX media are available in pre-packed preparative/process columns. With the exception of the 5 µm particle sizes, all materials are also available in bulk quantities. The standard pack sizes are 100 g and 1 kg, with larger quantities available for packing process columns up to 80 cm ID.

Agilent PLRP-S

	5 µm*	8 µm	10 µm	10-15 µm	15-20 µm	30 µm	50 µm
100Å	✓	✓	✓	✓	✓		✓
300Å	✓	✓	✓	✓	✓		✓
1000Å	✓	✓	✓			✓	✓
4000Å	✓	✓	✓			✓	

Agilent PL-SAX and PL-SCX

	5 µm*	8 µm	10 µm	30 µm
1000Å	✓	✓	✓	✓
4000Å	✓	✓	✓	✓

* The 5 µm particle sizes are recommended for high-efficiency laboratory prep with column dimensions of 25 mm ID and below.

Available preparative and process column dimensions

ID	Lengths		
	50 mm	150 mm	300 mm
4.6 mm	✓	✓	✓
7.5 mm	✓	✓	✓
25 mm		✓	✓
50 mm		✓	✓
100 mm			✓

Agilent PLRP-S columns

Dimensions (mm)	100Å	300Å	1000Å	4000Å
8 µm				
7.5 x 50		PL1112-1801	PL1112-1802	PL1112-1803
7.5 x 150		PL1112-3801	PL1112-3802	
7.5 x 300	PL1112-6800	PL1112-6801		
25 x 150	PL1212-3800	PL1212-3801		
25 x 300	PL1212-6800	PL1212-6801		
50 x 150	PL1712-3800	PL1712-3801		
50 x 300	PL1712-6800	PL1712-6801		
100 x 300	PL1812-6800	PL1812-6801		
10 µm				
25 x 50			PL1212-1102	PL1212-1103
25 x 150	PL1212-3100	PL1212-3101	PL1212-3102	PL1212-3103
25 x 300	PL1212-6100	PL1212-6101		
50 x 150	PL1712-3100	PL1712-3101	PL1712-3102	PL1712-3103
50 x 300	PL1712-6100	PL1712-6101		
100 x 300	PL1812-6100	PL1812-6101		
10-15 µm				
25 x 300	PL1212-6400	PL1212-6401		
50 x 150	PL1712-3400	PL1712-3401		
50 x 300	PL1712-6400	PL1712-6401		
100 x 300	PL1812-6400	PL1812-6401		
15-20 µm				
25 x 300	PL1212-6200	PL1212-6201		
50 x 150	PL1712-3200	PL1712-3201		
50 x 300	PL1712-6200	PL1712-6201		
100 x 300	PL1812-6200	PL1812-6201		
30 µm				
25 x 150			PL1212-3702	PL1212-3703
50 x 150			PL1712-3702	PL1712-3703
100x300			PL1812-3102	PL1812-3103

Agilent PL-SAX & PL-SCX columns

Dimensions (mm)	PL-SAX 1000Å	PL-SAX 4000Å	PL-SCX 1000Å	PL-SCX 4000Å
8 µm				
7.5 x 50	PL1151-1802	PL1151-1803	PL1145-1802	PL1145-1803
7.5 x 150	PL1151-3802	PL1151-3803	PL1145-3802	PL1145-3803
10 µm				
25 x 50	PL1251-1102	PL1251-1103	PL1245-1102	PL1245-1103
25 x 150	PL1251-3102	PL1251-3103	PL1245-3102	PL1245-3103
50 x 150	PL1751-3102	PL1751-3103	PL1745-3102	PL1745-3103
100 x 300	PL1851-2102	PL1851-2103	PL1845-2102	PL1845-2103
30 µm				
25 x 150	PL1251-3702	PL1251-3703	PL1245-3702	PL1245-3703
50 x 150	PL1751-3702	PL1751-3703	PL1745-3702	PL1745-3703
100 x 300	PL1851-3102	PL1851-3103	PL1845-3102	PL1845-3103

Agilent PLRP-S bulk media

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

Agilent PL-SAX & PL-SCX bulk media

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

Custom column and bulk media ordering

If you do not see the combination of pore/particle size and column dimensions or the bulk media quantity you are looking for in these tables, please contact your local sales office, who will assist you with our custom ordering process.

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