The Advantages of Polymeric PLRP-S Media

Technical Overview

Introduction

PLRP-S PS/DVB macroporous packings are inherently and uniformly hydrophobic, and do not need bonded ligands. All PLRP-S media are very robust and mechanically stable. The columns are widely used in separations of synthetic oligomers, synthetic polymer compositional analysis, gigaporous biomolecules, peptides, proteins and oligonucleotides.

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HPLC of peptides

Reversed-phase HPLC has become the method of choice for the analysis and purification of synthetic peptides. PLRP-S materials, with a range of optimized pore sizes, are ideally suited to this application area. Accessibility and high permeability of the molecules to the internal surface of the porous particle deliver excellent selectivity and capacity. The elevated chemical and physical stability of polymeric PLRP-S columns enable reproducible resolution with greatly exended column lifetimes, for thousands of injections over years of use. The packing is completely insoluble, and will not contaminate isolated fractions with leachable bonded phase. The ability to operate over the entire pH range and virtually all mobile phase compositions enables greater selectivity and unrestricted clean-up procedures. The high available surface area provides retention for even small peptides as demonstrated in (Figure 1).

Conditions

Column: PLRP-S 300Å 5 µm, 250 x 4.6 mm (p/n PL1512-5501)
Eluent A: 0.1% TFA in 20% ACN:80% Water
Eluent B: 0.1% TFA in 80% ACN:20% Water
Gradient: 0-100% B in 20 min
Flow Rate: 1 mL/min
Detection: UV, 220 nm

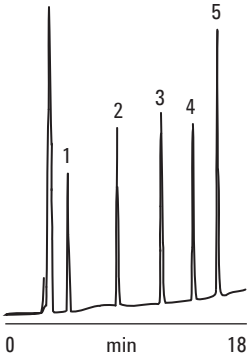

Figure 1. Poly(phenylalanine) separation (H-(Phe)$_n$-OH (n = 1, 2, 3, 4 or 5).

Absence of peak tailing

The excellent performance of PLRP-S with regard to peak tailing is evident in the separation of peptide standards (Figure 2).

Conditions

Sample: Soy protein isolated peptide standards
Column: PLRP-S and C18, 250 x 4.6 mm
Eluent A: 0.1% TFA in 1% ACN:99% Water
Eluent B: 0.1% TFA in 99% ACN:1% Water
Gradient: 10-30% B in 20 min
Flow Rate: 1 mL/min
Detector: UV, 220 nm

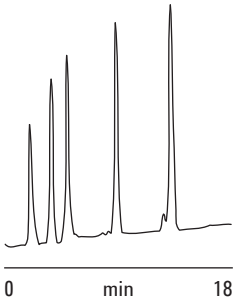

Figure 2. Superior performance from PLRP-S with no peak tailing.

Stability of the base matrix

The PLRP-S matrix is completely inert to conditions normally encountered during separation procedures. The physical stability of the rigid adsorbent is very high and should not be confused with other softer or porous polymeric particles. Additionally, the rigorous procedures we use during manufacture eliminate residual monomer and surfactant from the production processes. The physical stability and strength of the PLRP-S 300Å packing is demonstrated by the linear flow characteristics up to and over 5000 psi (345 bar) on analytical columns. In preparative 150 x 50 mm columns this allows continuous operation at flow rates over 200 mL/min and 700 mL/min for 10 µm and 20 µm packings, respectively, at only 1500 psi (Figure 3).

Conditions

Column: PLRP-S 300Å 10 µm or 20 µm, 150 x 50 mm
Eluent: 25% ACN:75% Water

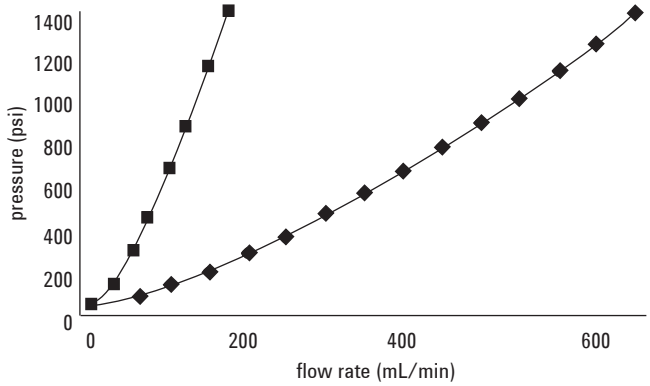

Figure 3. Pressure vs flow rate for two sizes of PLRP-S 300Å.

Relative hydrophobicity

The elution order of peptides on a PLRP-S column is generally similar to that of silica based columns (Figure 4).

Conditions

Column: 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 mL/min
Detection: UV, 220 nm

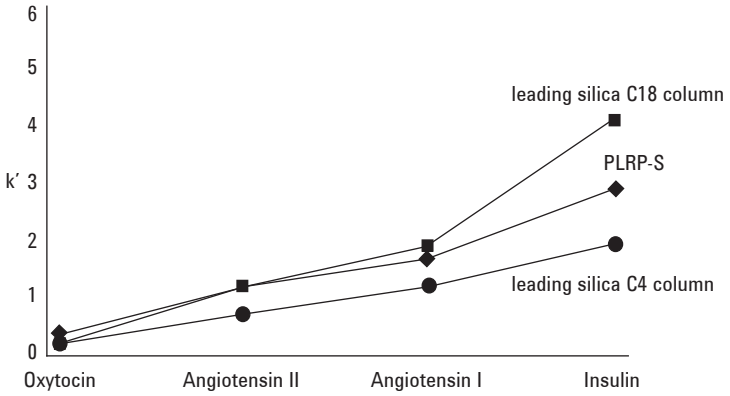

Figure 4. PLRP-S retention characteristics deliver excellent performance with a range of peptides.

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 polystyrene-based matrix. However, the observed capacity factor k' (calculated from the formula $k' = (t_R - t_\circ)/t_\circ$ where $t_\circ$ is the retention time for a non-sorbed solute) for four peptides on leading C18 and C4 silica columns and PLRP-S indicates the similarity of the interaction. The retention characteristics of the PLRP-S column suggest it would offer excellent performance over a wide range of peptides.

Surface area

Variation of pore size has a significant effect on the retention characteristics of peptides on silica and polymeric columns. Purification of peptides usually requires a pore size of 100Å or 300Å for large polypeptides in order to minimize restricted access or exclusion of larger molecules, but retaining the desired mass transfer properties for effective separation. Generally, the surface area of a 100Å or 300Å pore size silica is less than 200-300 m^2g^{-1}. The surface area of PLRP-S varies according to pore size (100, 300, 1000 and 4000Å, and the larger pore size material may be used to obtain satisfactory separations in a shorter period of time. However, loading capacity is affected proportionately.

PLRP-S pore size (Å)	Surface area (m^2g^{-1})
100	414
300	384
1000	267
4000	139

Column capacity

The actual amount of peptide which can be purified in one step is a key factor in peptide purification. Economy becomes increasingly important as flow rate, and therefore solvent consumption, increases and often preparative separations are performed under overload conditions. Band broadening is the primary cause of loss of resolution and introduces the practical limit to the amount of peptide which can be purified successfully. The absence of functionalities on polymeric columns is a significant advantage (Figure 5).

Conditions

Sample: ANF related fragment
Column: PLRP-S 300Å 10 µm, 4.6 x 150 mm (p/n PL1512-3101)
Eluent A: 0.1% TFA in 1% ACN:99% Water
Eluent B: 0.1% TFA in 99% ACN:1% Water
Gradient: 10% B for 5 min, 10-40% B in 15 min
Flow Rate: 1 mL/min
Detection: UV, 220 nm

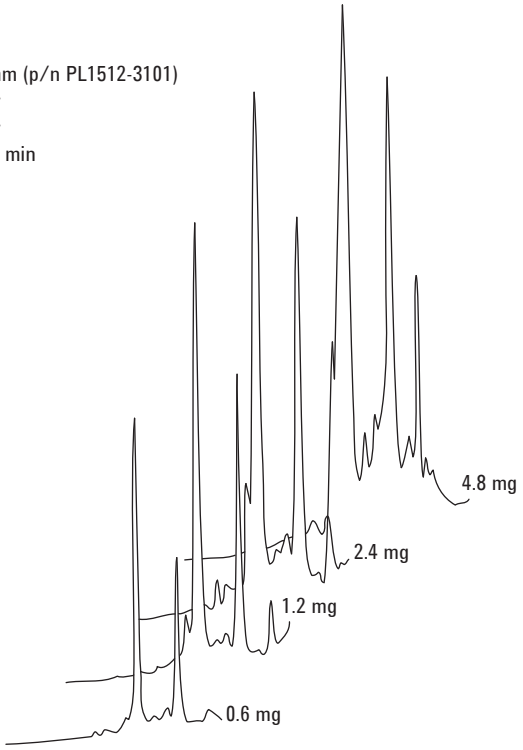

Figure 5. Loading capacity of a PLRP-S 300Å 10 µm column.

Utilization of pH stability

The availability of PLRP-S preparative columns with identical packing material enables the optimum loading capacity to be determined on an analytical scale. This reduces the likelihood of unnecessary losses during this vital stage of scale-up.

Since most peptides contain numerous acidic and basic residues, it is possible to significantly alter the retention characteristics of a peptide by adjusting the pH. For instance, the presence of a single aspartic acid residue in human angiotensin II (A) causes problems in the separation from angiotensin III (B) (Figure 6).

Peak Identification
A. H-Asp-Arg-Val-Tyr-Ile-His-Pro-Phe-OH
B. H-Arg-Val-Tyr-Ile-His-Pro-Phe-OH

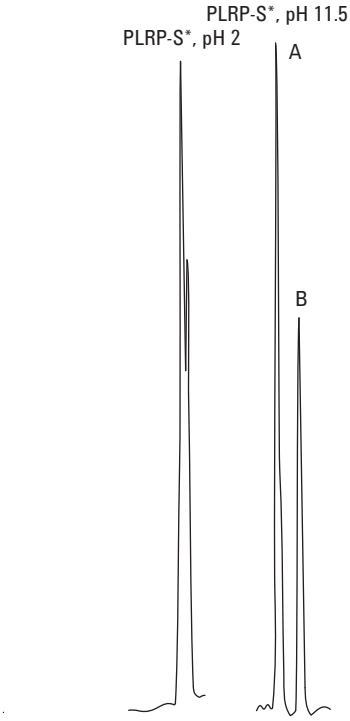

Figure 6. pH stability of PLRP-S in the separation of angiotensins (* after regular use).

Scale-up

Progressing from microgram to milligram or gram scale purification all too often involves re-developing the separation process to accommodate differing packing materials. Chromatographic process development can be performed using a PLRP-S analytical column and then applying the gradient and loading information directly to a larger preparative column packed with an identical material. This eliminates costly and time-consuming re-development steps which would otherwise be necessary (Figure 7).

Conditions - Column A

Sample: ANF related fragment
Column: PLRP-S 300Å 10 µm, 150 x 4.6 mm (p/n PL1512-3101)
Eluent A: 0.1% TFA in 1% ACN:99% Water
Eluent B: 0.1% TFA in 99% ACN:1% Water
Gradient: 15-95% B in 40 min
Flow Rate: 0.5 mL/min
Detector: UV, 220 nm

Conditions - Column B

Sample: ANF related fragment
Column: PLRP-S 300Å 10 µm, 150 x 25 mm (p/n PL1212-3101)
Eluent A: 0.1% TFA in 1% ACN:99% Water
Eluent B: 0.1% TFA in 99% ACN:1% Water
Gradient: 15-65% B in 50 min
Flow Rate: 7.5 mL/min
Detector: UV, 220 nm

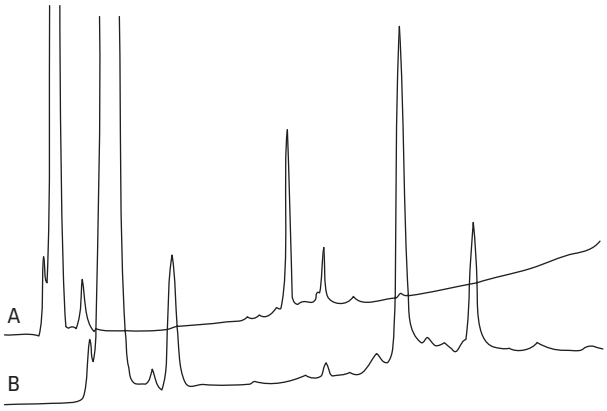

Figure 7. Separation on analytical (A) and preparative (B) columns packed with the same PLRP-S media.

These data represent typical results. For further information, contact your local Agilent Sales Office.

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Published in UK, July 15, 2011
5990-8435EN

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