

Agilent PLRP-S Media for HPLC Analysis of Peptides

Technical Overview

Introduction

Agilent PLRP-S is a rigid macroporous styrene/divinylbenzene (PS/DVB) HPLC phase with outstanding chemical and physical stability. PLRP-S HPLC media are inherently hydrophobic and reproducible, and do not require a bonded alkyl chain such as C8 or C18 to confer hydrophobicity. The columns are widely used in separations of synthetic oligomers, synthetic polymer compositional analysis, gigaporous biomolecules, peptides, proteins and oligonucleotides. All PLRP-S media are very robust and mechanically stable. In these examples we use PLRP-S for the analysis of peptides with high resolution.



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High resolution of peptide standards

The capability of PLRP-S is demonstrated in the resolution of five standard spi peptides. The mixture, RPS-P0010, produced by Synthetic Peptides Incorporated, is designed to monitor reversed-phase column performance. It contains five C-terminal amide decapeptides, four of which are N α -acetylated and the fifth contains a free N α -amino group (Figure 1).

Conditions

Column: PLRP-S 100Å 5 μ m, 250 x 4.6 mm (p/n PL1512-5500)
Eluent A: 0.1% TFA in 1% ACN, 99% Water
Eluent B: 0.1% TFA in 30% ACN, 70% Water
Gradient: Linear 0-100% B in 30 min
Flow Rate: 1.0 mL/min
Detection: UV, 220 nm

Peak Identification

1. Ala³-Gly⁴ (free amino)
2. Gly³-Gly⁴ (N α -acetylated)
3. Ala³-Gly⁴ (N α -acetylated)
4. Val³-Gly⁴ (N α -acetylated)
5. Val³-Gly⁴ (N α -acetylated)

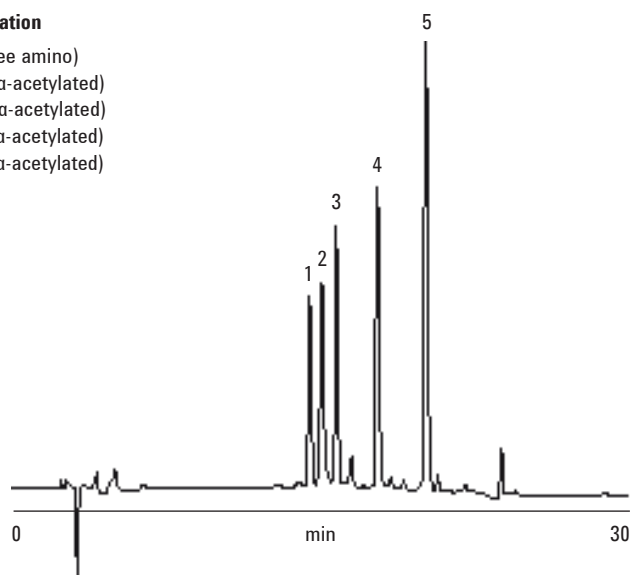


Figure 1. Separation of five spi peptide test probes on Agilent PLRP-S.

Selectivity in peptide RP-LC

The pore size of the PLRP-S 100Å material is optimized for the separation of peptides. The capability of the packing is demonstrated in the selectivity of peptides by reversed-phase LC. Separation of synthetic peptides indicates that the selectivity of PLRP-S is similar to that of alkyl bonded phases.

Conditions

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5500)
Eluent A: 0.1% TFA/1% 2-Propanol/Water
Eluent B: 0.1% TFA/1% 2-Propanol/ACN
Gradient: 95% A (0-3 min) to 50% A (13 min)
Flow Rate: 1.0 mL/min
Inj Vol: 100 µL
Detection: UV, 220 nm

Peak Identification

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

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

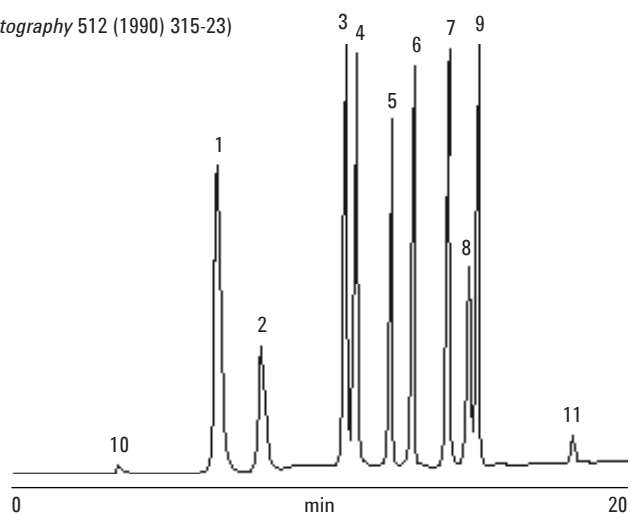


Figure 2. Good separation of peptide standards on Agilent PLRP-S.

Tryptic fingerprinting

The capability of PLRP-S is also demonstrated in the fingerprinting of tryptic peptides. Reversed-phase separation of tryptic peptides of species cytochrome C on PLRP-S 100Å reveals that slight differences in the amino acid sequence can be easily detected (Figure 3).

Conditions

Column: PLRP-S 100Å 5 µm, 250 x 4.6 mm (p/n PL1512-5500)
Eluent A: 0.1% TFA/1% 2-Propanol/Water
Eluent B: 0.1% TFA/1% 2-Propanol/ACN
Gradient: 95% A (0-5 min) to 60% A (55 min)
Flow Rate: 1.0 mL/min
Inj Vol: 35 µg in 100 µL
Detection: UV, 220 nm

Peak Identification

1. Tuna cytochrome C
2. Horse cytochrome C
3. Rabbit cytochrome C

(reproduced with permission from *J.Chromatography* 512 (1990) 315-23)

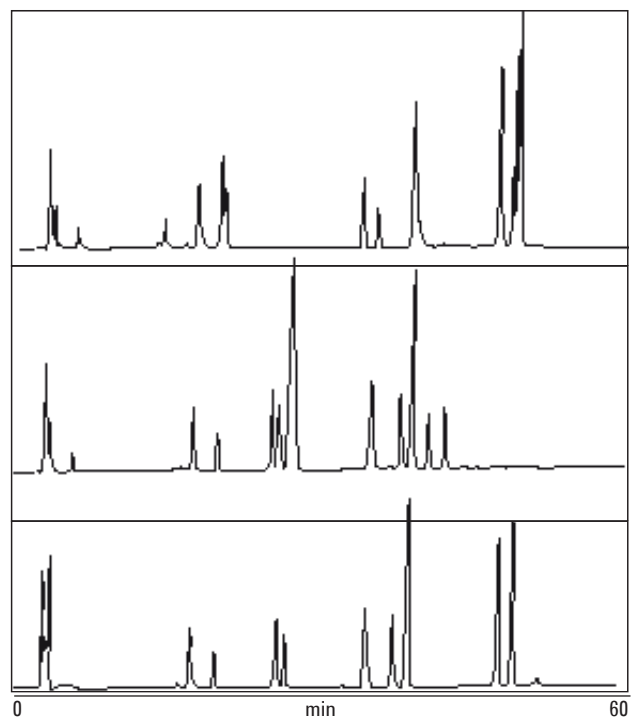


Figure 3. Agilent PLRP-S reveals the slight differences in amino acid sequences to facilitate tryptic fingerprinting.

PLRP-S, the complete range of rigid PS/DVB materials for HPLC

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

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

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