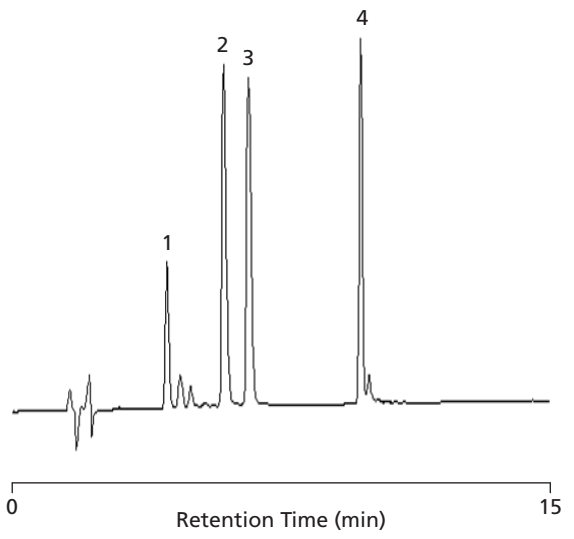
High Resolution/Fast RP-HPLC Analysis of Synthetic Peptides

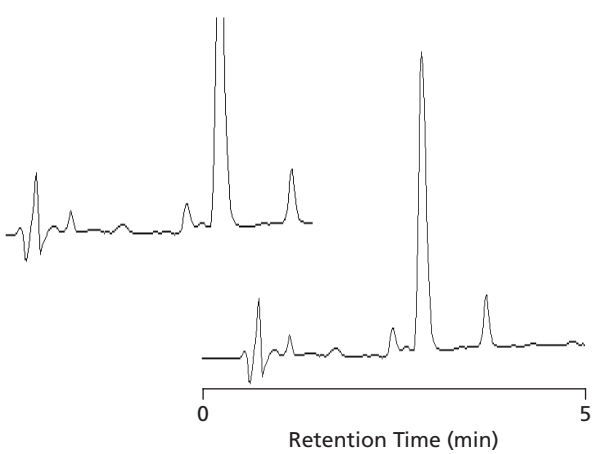
Using 3 µm Polymeric Particles

Figure 1: High-resolution separation of a mixture of peptide standards. Column: PLRP-S 100 Å 3 µm 150 × 4.6 mm i.d.; eluent A: 0.1% TFA in 20% ACN; eluent B: 0.1% TFA in 50% ACN; linear gradient: 0-100% B in 15 min, flow-rate: 1.0 mL/min.



Peaks: 1 = oxytocin, 2 = angiotensin II, 3 = angiotensin I, 4 = insulin.

Figure 2: High-speed screening of a synthetic peptide, peptide Ac-Lys-Tyr-Ala-Leu-Lys-Ala-Leu-Lys-Gly-Leu-Lys-acid. Column: PLRP-S 100 Å 3 µm 50 × 4.6 mm i.d.; eluent A: 0.1% TFA in 1% ACN; eluent B: 0.1% TFA in 50% ACN; linear gradient: 40-80% B in 5 min; flow-rate = 1.0 mL/min.



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Introduction

A new 3 µm rigid polymeric particle with an exceptionally narrow particle size distribution has been developed to complement the existing range of analytical, preparative and process PLRP-S reversed-phase materials. Two pore sizes are available, 100 Å for peptide analysis and 300 Å with improved mass transfer characteristics for large polypeptides and globular proteins.

Experimental Conditions

Reversed-phase HPLC was performed using a binary Knauer high-pressure mixing gradient system with variable wavelength UV/vis operated at 220nm (Knauer GmbH, Germany). PLRP-S 100 Å 3 µm particles were packed using a high-pressure slurry reservoir technique in 150 × 4.6 mm i.d. and 50 × 4.6 mm i.d. columns. The chromatographic conditions are detailed in the figure legends.

Results

PLRP-S 100 Å 3 µm particles are packed in 150 mm column lengths for applications, which require high resolution. Conventional 4.6 mm i.d. columns are used where sensitivity and sample availability are not an issue. For high-resolution analyses of small sample volumes, 2.1 mm and 1.0 mm i.d. columns are available.

Figure 1 shows a separation of four peptide standards using the 4.6 mm i.d. column. The selectivity is the same as the larger PLRP-S 100 Å particles but the increased efficiency provides improved resolution of the minor components. For reduced analysis times, shorter column lengths are ideal and therefore, the 3 µm particles are also available in a 50 mm column length and 4.6 mm, 2.1 mm and 1.0 mm i.d..

Figure 2 shows a separation of a crude synthetic peptide screened using a 5 min gradient. The resolution of the closely related contaminants can clearly be seen when the y-axis is expanded.

Conclusions

The high efficiency PLRP-S 3 µm particles complement the family of PLRP-S analytical and prep/process media providing high resolution and/or short analysis times. The chemical stability and zero particle bleed make these media ideal for use as a universal RP-HPLC column for peptide analysis and purification include LC-MS applications.

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