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The Vanguard of Liquid Chromatography.

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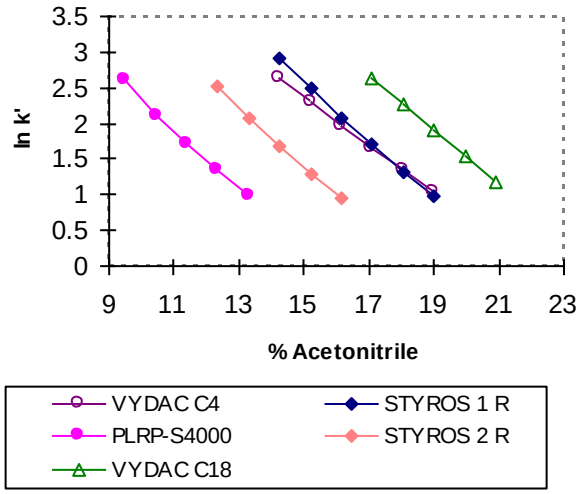
APPLICATION NOTE

Comparison of STYROS™ 1R and 2R with Silica C4 Stationary Phase.

Most of the reversed-phase analytical separation methods have been developed on Silica C4 stationary phases. In the last several years, numerous companies have introduced macroporous poly (styrene-divynilbenzene) (PS-DVB) media to replace silica. The polymeric stationary phases provided the advantage of high chemical stability, but they had several shortcomings, namely low pressure tolerance (<3000 psi) and low capacity (5-10 mg/ml Lysozyme).
We have addressed these deficiencies during the development of the first two generations of STYROS™ chromatographic media family. The retentivity of the two new media is compared, in Figure 1, to that of PLRP-S 4000 [1] (PS-DVB) and Vydac[2] C4 (silica based aliphatic ligand) using Angiotensin III. The retentivity of STYROS™ 1R closely matches that of C4 while the retentivity of STYROS™ 2R is in between the C4 and PLRP-S. Both STYROS™ stationary phases can tolerate pressure up to 5,000 to 10,000 psi without irreversibly collapsing

Figure 1. Retention map of Angiotensin III on different stationary phases



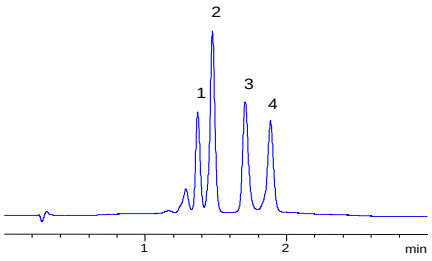
Standard protein and peptide separations on STYROS™ 1 R and on VYDAC C4 have been compared in chromatograms 1-4.
Chromatograms 1 and 2 show the selectivity of the two columns to be practically the same for proteins.

STANDARD PROTEIN SEPARATIONS

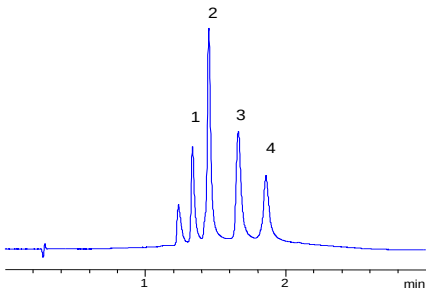
Table 1. HPLC Operating Parameters for Chromatograms 1 and 2.

HPLC System	Hewlett Packard 1100
Detector	214 nm
Column	STYROS™ 1 R/XH 50x4.6mm (Chromatogram 1). VYDAC C4 50x4.6 mm (Chromatogram 2).
Mobile Phase	A: 0.1 % TFA in water B: 0.1 % TFA in Acetonitrile/water (95/5)
Gradient	15-80% B in 2 minutes
Flow rate	2.5 ml/min
Temperature	Ambient
Injection volume	10 µl
Sample: **1 mg/ml each**	1: Cytochrome C, 2: Lysozyme 3: β-Lactoglobulin, 4: Ovalbumin

Chromatogram 1: Standard protein separation on STYROS™ 1 R/XH



Chromatogram 2: Standard protein separation on VYDAC C4



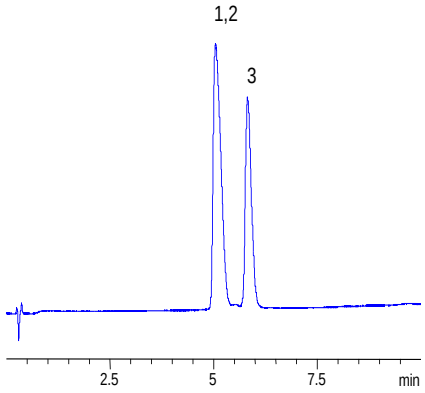
[1] From Polymer Laboratories, UK
[2] From The Separation Group, Hesperia, California, USA

SEPARATION OF ANGIOTENSIN VARIANTS

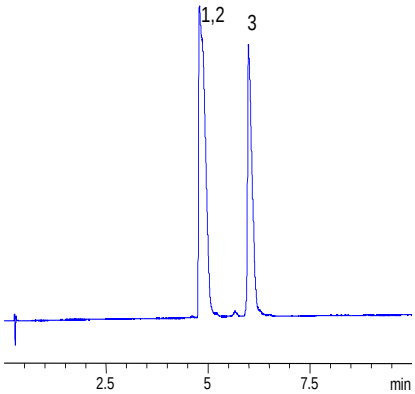
Table 2. HPLC Operating Parameters for Chromatograms 3 and 4.

HPLC System	Hewlett Packard 1050
Detector	214 nm
Column	STYROS™ 1 R/XH 50x4.6mm (Chromatogram 3) VYDAC C4 50x4.6 mm (Chromatogram 4)
Mobile Phase	A: 0.1 % TFA in water B: 0.1 % TFA in Acetonitrile/water 95/5
Gradient	5-40% B in 10 minutes
Flow rate	1 ml/min
Temperature	Ambient
Injection volume	10 µl
Sample **1 mg/ml each**	1: Angiotensin II 2: Angiotensin III 3: Angiotensin I

Chromatogram 3: Separation of Angiotensin Variants on STYROS™ 1 R/XH



Chromatogram 4: Separation of Angiotensin variants on VYDAC C4



The selectivity of the two columns is slightly different towards the Angiotensin variants (chromatograms 3 and 4). In general, the peaks are less tailing on STYROS™ than on VYDAC C4, most probably due to the absence of silanol groups. Similarly to numerous other peptides, Angiotensin II and III can be separated only under basic conditions as depicted in chromatogram 6. However, silica based media can not be used above pH 8.

SEPARATION OF ANGIOTENSIN VARIANTS AT BASIC pH.

Table 3. HPLC Operating parameters for Chromatogram 5.

HPLC System	Hewlett Packard 1050
Detector	214 nm
Column	STYROS™ 1 R/XH 100x4.6mm
Mobile Phase	A: 10 mM phosphate in water, pH 11.2 B: Acetonitrile
Gradient	0-40% B in 25 ml
Flow rate	2.5 ml/min
Temperature	Ambient
Injection volume	10 µl
Sample 1 mg/ml each	1: Angiotensin II, 2: Angiotensin III 3: Angiotensin I

Chromatogram 5. Separation of Angiotensin Variants on STYROS™ 1 R/XH at 2.5 ml/min, pH 11.2