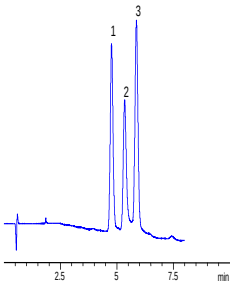
OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: ***info@orachrom.com*** ***www.orachrom.com***

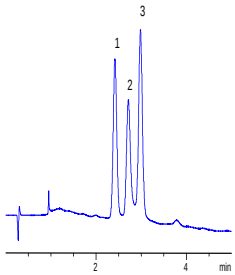
APPLICATION NOTE

Separation of Angiotensin Variants at Basic pH.

It is well established that the separations of Angiotensins II and III is not possible on reversed-phase stationary phases at acidic pH.
In order to achieve such separation, which also holds true for numerous other peptides of similar structure, it is necessary to operate at relatively high pH (>10) where the use of silica based media is not possible.
The present application note provides an example where the use of **STYROS** ™ becomes a convenient tool to operate at high pH and resolve the separation.
The optimized pore structure of the polymeric matrix that makes up the core of **STYROS** ™**1** and **STYROS** ™**2** can not only operate in full pH range, it also makes it possible to maintain high resolution even at high speed. The following chromatograms highlight such characteristic.



Chromatogram 1. Separation of Angiotensin variants at pH=11.2, on **STYROS**™ **1 R**/XH at 900 cm/hr.

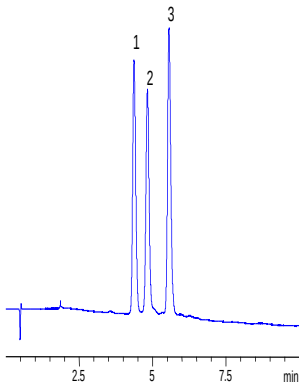


Chromatogram 2. Separation of Angiotensin variants at pH=11.2, on **STYROS** ™ **1 R**/XH at 1,800 cm/hr.

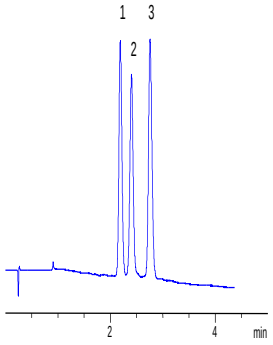
Table 1. Operating Parameters for Chromatograms 1 & 2.

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 and 5 ml/min
Temperature	Ambient
Injection volume	10 µl
Sample **1 mg/ml each**	1: Angiotensin II, 2: Angiotensin III 3: Angiotensin I

STYROS ™ **2** provides a different surface chemistry within the same optimized pore structure. As a result, while maintaining a similar high performance at low and high flow rates, it is possible to get different selectivity and retentivity by using **STYROS** ™ **2**.



Chromatogram 3. Separation of Angiotensin variants at pH=11.2, on **STYROS** ™ **2 R**/XH at 900 cm/hr.



Chromatogram 4. Separation of Angiotensin variants at pH=11.2, on **STYROS** ™ **2 R**/XH at 1,800 cm/hr.

Table 2. Operating Parameters for Chromatograms 3 & 4.

HPLC System	Hewlett Packard 1050
Detector	214 nm
Column	**STYROS** ™ **2 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 and 5 ml/min
Temperature	Ambient
Injection volume	10 µl
Sample **1 mg/ml each**	1: Angiotensin II, 2: Angiotensin III 3: Angiotensin I