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

High Performance Separation of Proteins on STYROS™ R/NB.

The demand of Narrow Bore columns for high performance separations has become an important issue in light of the high frequency of sample run with the new generation of automated HPLC instruments.
The concern over the consumption of large volume of solvents as a consequence of such frequent use, as well as the depletion of high value sample, especially during method development, has moved the researcher into smaller volume columns where excessive back pressure have restricted the option of high speed.
To address this major concern, it was necessary to conceive of a media that could tolerate the high back pressure of packing without altering the optimized pore structure and still provide the type of back pressure that allows the column to be run at high flow rates. In addition to the pressure tolerance, significantly higher capacities were needed to compensate for the column volume reduction as a consequence of smaller diameter.
The first two STYROS™ reversed-phase media that OraChrom has made available to the chromatographer satisfy such requirements.
Figure 1 shows a set of 3 chromatograms comparing the separation of a complex mixture of 5 proteins (Soybean Trypsin Inhibitor (STI), Cytochrome C (Cyt C), Lysozyme (Lys), Hemoglobin (Hemo), and Ovalbumin (OVA).

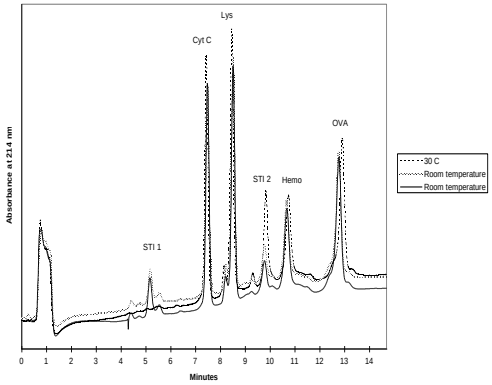

Figure 1. Separation of Standard Protein Mixture on a STYROS™ 1 R/NB (250x2.1 mm) at 1,700 cm/hr.

The chromatograhic conditions are summarized in the following table.

System	Waters 2690
Column	STYROS™ 1 R/NB 250x2.1 mm
Mobile Phase	A: 0.1% (v/v) TFA in water B: 0.1% (v/v) TFA in Acetonitrile/Water 95/5 (v/v)
Flow rate	1 ml/min (1700 cm/hr) (Figure 1) 2 ml/min (3400 cm/hr) (Figure 2)
Gradient	15% B to 80% B in 20 ml
Temperature	25 and 30 °C (Figure 1) 60 °C (Figure 2)
Sample volume	1 µl
Detection	214 nm
Sample 1 mg/ml each	Soybean Trypsin Inhibitor (STI), Cytochrome C (Cyt C), Lysozyme (Lys), Hemoglobin (Hemo), Ovalbumin (OVA)

It is important to note that such separation would have required 75minutes instead of 15 at the traditional linear flow rate of 340 cm/hr. The STYROS ™ column was reequilibrated at 3 ml/min (5,100 cm/hr) with 17 column volume during 5 minutes. A similar reequilibration would have required one hour and 16 minutes at 0.2 ml/min of flow rate.
Due to the stable nature of the polymeric matrix it is also possible to operate at higher temperatures. Figure 2 shows the chromatogram of the same mixture at 60 °C and 3,400 cm/hr.

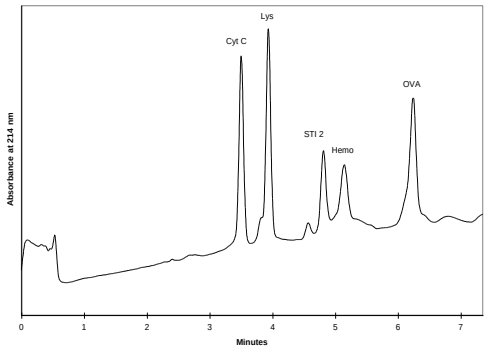

Figure 2. Separation of Standard Protein Mixture on a STYROS ™ 1 R/NB (250x2.1 mm) at 3,400 cm/hr and 60 °C.

Operating in such mode, the run time is reduced in half without compromising the resolution. The added benefit of speed variation and therefore control of the residency time of the biomolecules on the stationary phase, makes it possible to monitor different forms of the protein. STI is a known example of protein to unfold during reversed-phase chromatography. The present chromatograms show the folded part of the protein eluting at ≅ 5 minutes in figure 1, to fully subside to the unfolded part (≅ 10 minutes) as the temperature increases. Similar phenomenon can be observed by reducing the flow rate.
The use of the Narrow Bore STYROS™ of different lengths is also widespread in hyphenated techniques such as LC/MS where the polymeric nature of the stationary phase provides an optimum matrix to explore the use of buffer ingredients that would interfere least with the resulting mass fragments.