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

STYROS® HQ, Strong Anion Exchanger, Compared with STYROS® 1R Reversed Phase Polymeric. A line of Stable Polymeric Simulated-Monolith™ to Replace Slow and Leaching Soft Gels.

In this Application Note we have compared two polymeric stable media to find out the differences and therefore the ample choices they provide in the separation of proteins in biologicals.

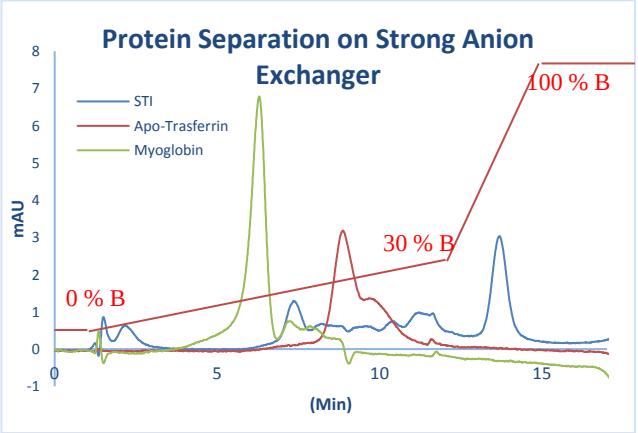

Table 1. Operating parameters.

HPLC System.	Agilent 1260 with thermostatted column compartment and quaternary pump.
Columns	**STYROS® HQ** 2.1 X 100 mm (0.346 ml volume)
Mobile phase.	A: 20 mM Bis-Tris, pH=6 B: A + 1 M NaCl, pH= 6
Flow rates	0.2 ml/min (347 cm/hr of linear velocity)
Gradient	0 % B for 1 min to 30 % B in13 min to 100 % B in 15 min.
Temperature	30°C
Detection	280 nm
Injection volume	0.5-2 µl
Pressure Drop	3 bars
Sample:	Myoglobin, apo-Transferrin, Soybean trypsin inhibitor.

Notice the low pH needed to operate the weak anion exchanger as in addition to the positive charge generated by the low pH it also has hydrophobic entities involved in the separation.

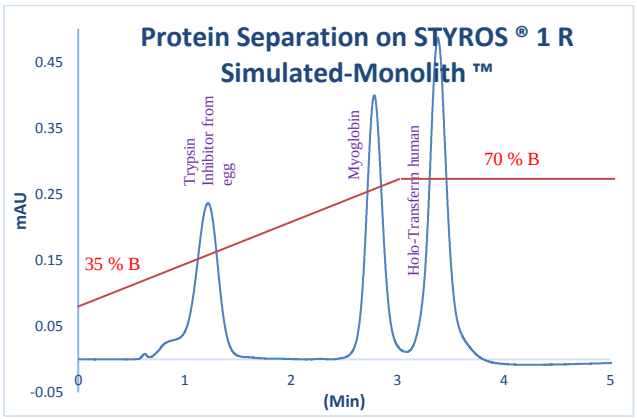

Table 2. Operating parameters.

HPLC System.	Acquity UPLC *I Class Plus*
Column	**STYROS® 1R** 2.1 X 50 mm (0.173 ml volume)
Mobile phase.	A : 2% ACN in DI H2O, 0.1 % TFA B: 70:30 ACN: H2O, 0.1 % TFA
Flow rates	0.2 ml/min (>300 cm/hr of linear velocity)
Gradient	35 to 70 % B in 3 min
Temperature	30°C
Detection	220 nm
Injection volume	2 µl
Pressure Drop	< 100 psi on the column
Sample:	5 mg/ml of proteins in A.

These stationary phases operate like monolith but are not prone to the "wall effects" and leaching, that monolithic media suffer from. They are identified as Simulated-Monolith™.

More importantly unlike soft gel and most other chromatographic media on the market, they are stable polymerics and they do not leach. Such advantage is essential in the downstream processes of vaccines, among other biopharmaceuticals that heavily depend on high purity that even "Polishing" cannot provide.

Inc.