

OraChrom, Inc.

The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA

Phone (781) 932 0151
E-mail: info@orachrom.com

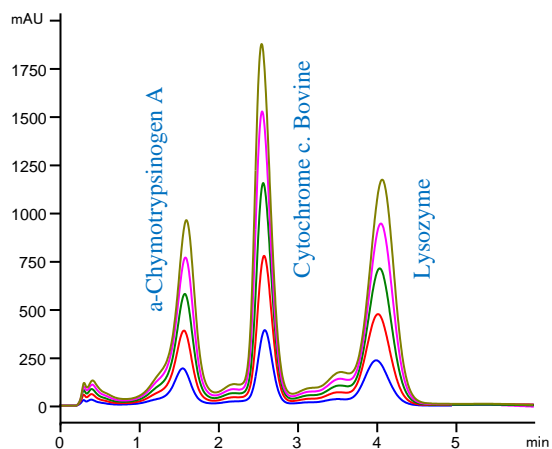
Fax (781) 932 0787
www.orachrom.com

APPLICATION NOTE

STYROS™ SP Simulated Monolith™ Strong Cation Exchangers: Loadability and Linear Velocity.

It is critical in the downstream process of biopharmaceutical to have access to resins with high capacity, high speed, very high resolution, high salt tolerance, low back pressure as well as non-leaching media that can be used many times over to justify not only its cost but can also save by allowing CIP procedure in a fast and efficient way.

STYROS™ SP/XH Simulated Monolith™, a high capacity cation exchanger based on hard gel polymeric with over 100 mg/ml Lysozyme, is shown here with some of its characteristics.



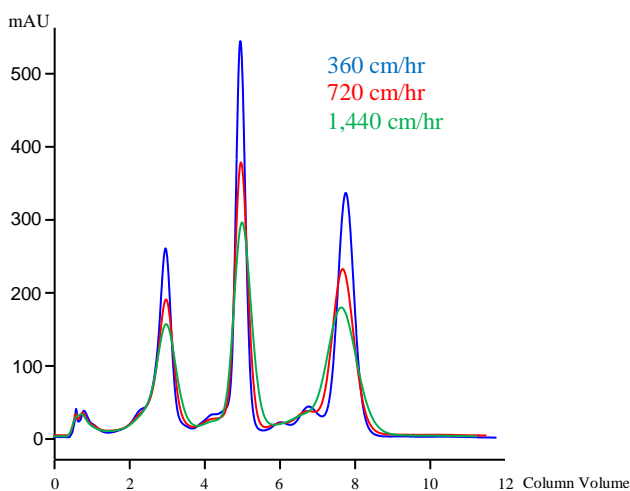
Chromatogram 1
Loadability study at 720 cm/hr on a 4.6 x 50 mm STYROS™ SP/XH.

Table 1. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	STYROS™ SP/XH 4.6 x 50 mm
Mobile Phase	A: 25 mM MES, pH = 6.2 B: A + 1 M NaCl, pH = 6.2
Flow rate	2 ml/min (720 cm/hr)
Gradient	12 to 60 % B in 12 cv.
Temperature	30°C
Detection	280 nm
Injection volume	20, 40, 60, 80 and 100 µl
Pressure Drop	12 bar (174 psi)
Samples	α-Chymotrypsinogen A, Cytochrome C from Bovine, Lysozyme. 5mg/ml each.

The dynamic capacity is also affected by linear velocity which does change the resolution of the separation. In order to speed up the process the manufacturer needs to operate at the highest flow rate possible.

In the following chromatogram the media has been tested at high flow rates while the resolution and the pressure drop have been monitored.



Chromatogram 2
Resolution Variability at Higher Flow Rates of up to 1,400 cm/hr.

Table 2. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	STYROS™ SP/XH 4.6 x 50 mm
Mobile Phase	A: 25 mM MES, pH = 6.2 B: A + 1 M NaCl, pH = 6.2
Flow rate	1, 2 and 4 ml/min (360,720 and 1,440 cm/hr)
Gradient	12 to 60 % B in 12 cv.
Temperature	30°C
Detection	280 nm
Injection volume	20 µl
Pressure Drop	4, 8 and 16 bar (58, 116 and 232 psi)
Samples	α-Chymotrypsinogen A, Cytochrome C from Bovine, Lysozyme 5mg/ml each.

The buffer pH is also a parameter that can influence the separation.

