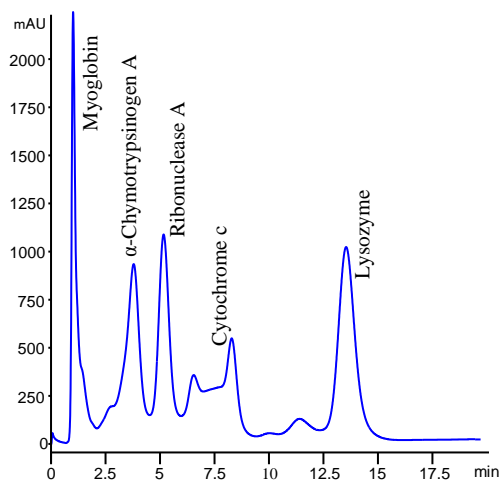


## APPLICATION NOTE

### STYROS™ SP Simulated Monolith™ Strong Cation Exchanger Compared to CM Simulated Monolith™ Weak Cation Exchanger. Sequence of Protein Elution.

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 also the time saved by allowing CIP procedures in a fast and efficient way.

**STYROS™** Simulated Monolith™ cation exchangers are offered in both high capacity strong cation exchangers (SP) and lower capacity weak cation exchanger (CM). These are based on hard gel polymeric with over 100 mg/ml and 50 mg/ml of Lysozyme capacity respectively.

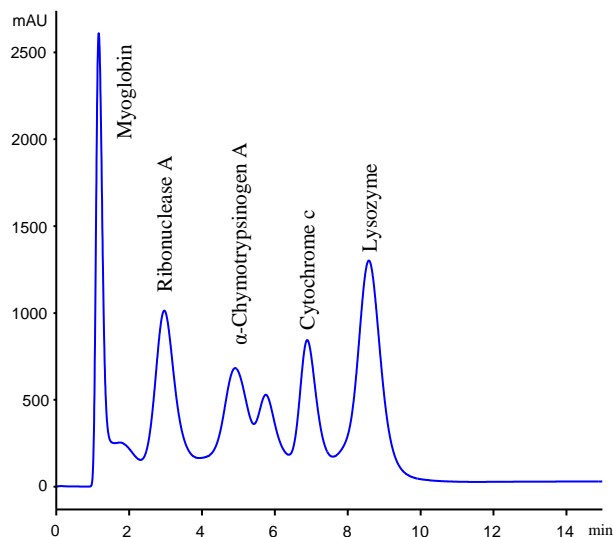


Chromatogram 1

Separation of 5 proteins on a 4.6 x 100 mm STYROS™ SP/XH.

**Table 1. Operating Parameters.**

<b>HPLC System.</b>	HP 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™</b> SP/XH 4.6 x 100 mm
<b>Mobile Phase</b>	A: 20 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
<b>Flow rate</b>	1 ml/min (360 cm/hr)
<b>Gradient</b>	12 to 55 % B in 12 cv.
<b>Temperature</b>	30°C
<b>Detection</b>	214 nm
<b>Injection volume</b>	20 µl
<b>Pressure Drop</b>	9 bar (131 psi)
<b>Samples (1:3:3:3:3 mg/ml each)</b>	Myoglobin, α-Chymotrypsinogen A, Ribonuclease A, Cytochrome C from equine, Lysozyme.



Chromatogram 2

Separation of 5 proteins on a 4.6x100mm STYROS™ CM/XH

**Table 2. Operating Parameters.**

<b>HPLC System.</b>	HP 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™</b> CM/XH 4.6 x 100 mm
<b>Mobile Phase</b>	A: 20 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
<b>Flow rate</b>	1 ml/min (360 cm/hr)
<b>Gradient</b>	3 to 35 % B in 9 cv.
<b>Temperature</b>	30°C
<b>Detection</b>	214 nm
<b>Injection volume</b>	20 µl
<b>Pressure Drop</b>	6 bar (87 psi)
<b>Samples (1:3:3:3:3 mg/ml each)</b>	Myoglobin, Ribonuclease A, α-Chymotrypsinogen A, Cytochrome C from equine, Lysozyme.

Note the effects of ionic strength as well as high capacity on peak retention reversal as well as the discriminatory property of the strong cation exchanger particularly on Cytochrome c and weak cation exchanger on α-Chymotrypsinogen A.

