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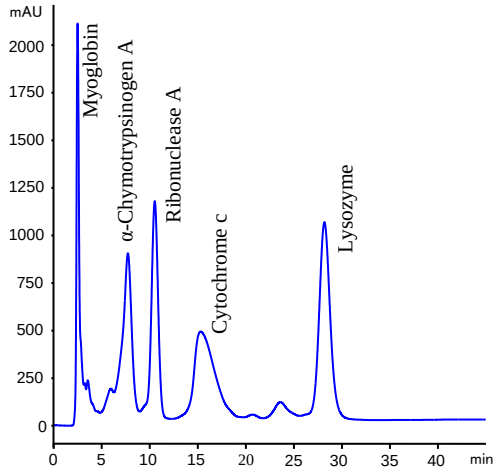
APPLICATION NOTE

STYROS™ SP Simulated Monolith™ Strong Cation Exchangers: Longer Columns for Added Resolution.

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.

Low back pressure is an important feature that allows the possibility of having longer columns for additional resolution.
In this application note we have compared a 10 cm column of 4.6 mm ID with a 30 cm column of 4 mm ID.

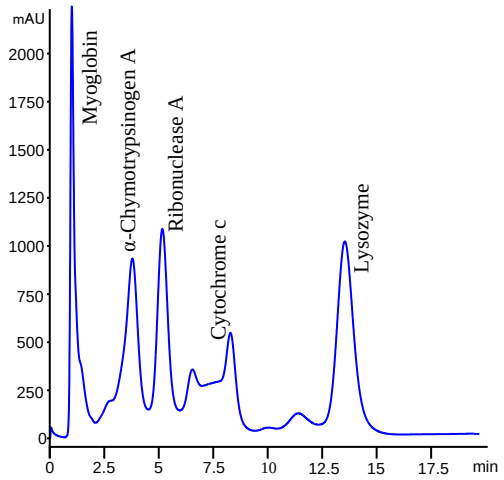


Chromatogram 1
Separation of 5 proteins at 480 cm/hr on a 4 x 300 mm STYROS™ SP/XH.

Table 1. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	**STYROS™** SP/XH 4 x 300 mm
Mobile Phase	A: 20 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
Flow rate	1 ml/min (478 cm/hr)
Gradient	12 to 55 % B in 12 cv,
Temperature	30°C
Detection	214 nm
Injection volume	30 µl
Pressure Drop	24 bar (348 psi)
Samples 1:3:3:3:3	Myoglobin, α-Chymotrypsinogen A, Ribonuclease A, Cytochrome c, Lysozyme in buffer A.

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.
Although a smaller column can provide adequate resolution in half the time, there is still room for added resolution using a longer column.



Chromatogram 2
Separation of 5 proteins at 360 cm/hr on a 4.6 x 100 mm STYROS™ SP/XH.

Table 2. Operating Parameters.

HPLC System.	HP 1100 with thermostatted column compartment.
Columns	**STYROS™** SP/XH 4.6 x 100 mm
Mobile Phase	A: 25 mM Phosphate, pH = 6.8 B: A + 1 M NaCl, pH = 6.8
Flow rate	1 ml/min (360, cm/hr)
Gradient	12 to 55 % B in 12 cv.
Temperature	30°C
Detection	214 nm
Injection volume	20 µl
Pressure Drop	9 bar (131 psi)
Samples 1:3:3:3:3	Myoglobin, α-Chymotrypsinogen A, Ribonuclease A, Cytochrome c, Lysozyme in buffer A.

Inc.