OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

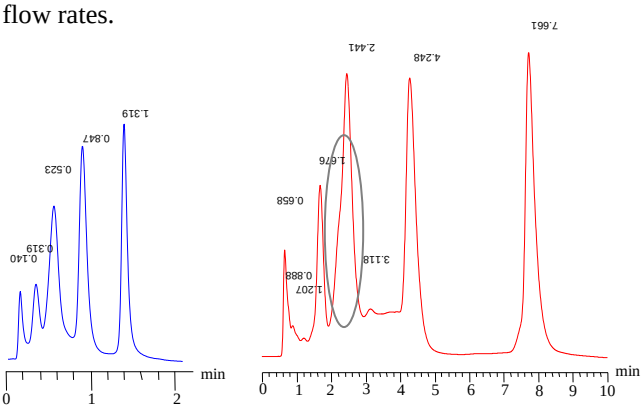
Resolving Efficiency of Longer Columns: case application with medium capacity cation exchanger.

Chromatographic columns with a wide range of mechanical and chemical stability provide the possibility of being used within a wide range of conditions without any restrictions.

When column length remains the only parameter to achieve resolution, hard gel chromatographic media such as **STYROS**™ offer an additional advantage over soft gel.

The following examples show the separation of 4 proteins at different flow rates and the effect of column length on resolution.

The columns are packed with **STYROS**™ SE, a medium capacity strong cation exchanger and run at two different flow rates.



STYROS™SE/XH
Strong Cation Exchanger
4.6 x 33 mm
5 ml/min (1,800 cm/hr)

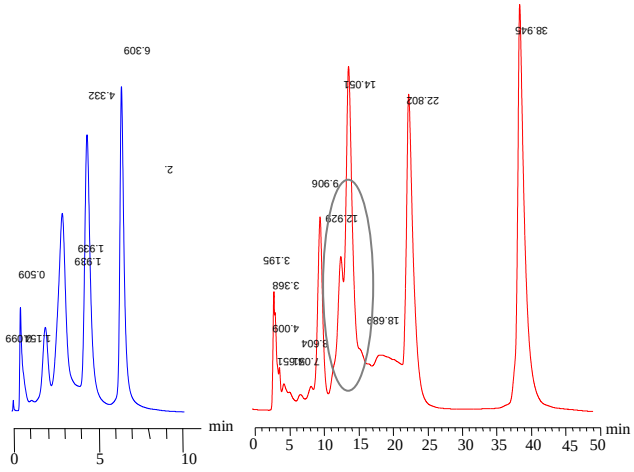
STYROS™SE/XH
Strong Cation Exchanger
4.6 x 250 mm
5 ml/min (1,800 cm/hr)

Table 1. Operating Parameters.

HPLC System.	HP 1100
Columns	**STYROS**™ SE/XH 4.6 x 33 mm and 4.6 x 250 mm
Mobile Phase	A: 20 mM Phosphate, pH = 7 B: A + 1 M NaCl, Ph=7
Flow rate	5 ml/min (1,800 cm/hr) and 1 ml/min (360 cm/hr)
Gradient	5 to 15 % B in 7.5 cv, to 60 % B in 12 cv.
Temperature	30°C
Detection	280 nm

Injection volume	3.3 µl in 33 mm column, 25 µl in 250 mm column
Samples **4 proteins**	Ribonuclease A, -Chymotrypsinogen, Cytochrome C, Lysozyme (5:5:5:4 mg/ml).

Although preliminary separations could be achieved at high linear velocities with both the 3.3 and 25cm columns, the longer column displays a clear shoulder at 2.4 min retention time that is not present in the separations with the 3.3 cm column.
Further reduction of the speed, from 1,800 to 360 cm/hr, reveals a clear peak at 12.9 min during the separation with the 25cm column. The smaller column would still not be able to resolve the isoform of the protein.



STYROS™SE/XH
Strong Cation Exchanger
4.6 x 33 mm
1 ml/min (360 cm/hr)

STYROS™SE/XH
Strong Cation Exchanger
4.6 x 250 mm
1 ml/min (360 cm/hr)

The back pressure of the 25 cm column at 5 ml/min flow rate does not exceed 120 bar (~ 1740 psi).
The column can withstand up to 4000 psi without irreversibly collapsing. Such characteristics provide the chromatographic media the advantage of speed as well as bed length over conventional soft gel media, without the loss of resolution.

Notice the linear velocity of 1,800 cm/hr (based on an empty column), far exceeds those used with either soft gel or non pervious media.