OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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APPLICATION NOTE

Hydrophobic Interaction Chromatography: With Limited Sample.

With the understanding of HIC mode of separation (see Application Note AN-081812-67 and additional ones on our web site) it is now possible to choose the right column dimensions from the list of available columns to address different separation cases.
Every restriction that the stationary phase imposes on the end user is an impediment on the work and therefore negatively impacts the outcome of the separation and purification.

When the amount of supply is limited or of high cost and the generation of waste is factored in the budget, the end user has the option of smaller yet still high resolving columns.

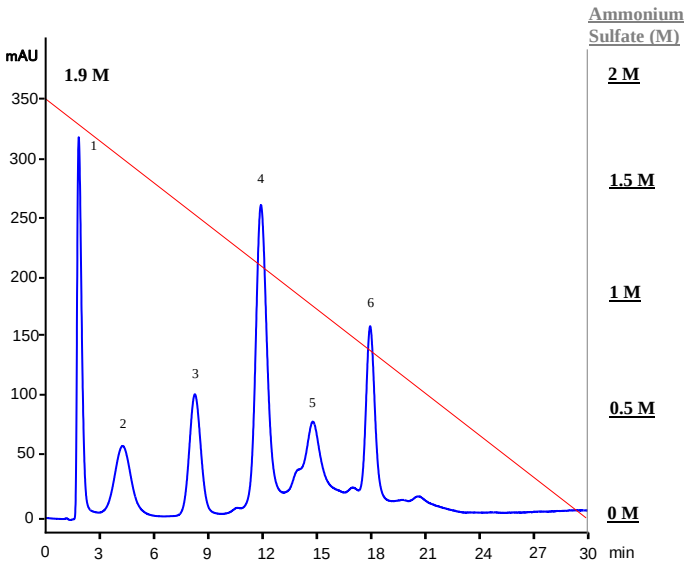

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HIC-Butyl/NB** 2.1 X 300 mm(v=1.04ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO4(NH4)2, pH=7
Flow rate	0.5 ml/min (870 cm/hr)
Gradient	90 to 0 % B in 30 min (14.5 cv)
Temperature	30°C
Detection	214 nm
Injection volume	3 µl
Sample:	1-Cytochrome c, 2-Myoglobin, 3-Ribonuclease A , 4-Lysozyme, 5- Ovalbumin , 6- α-Chymotrypsinogen A 1 mg/ml each in buffer A.

The column used in the present case is a narrow bore column of 2.1mm ID.
The amount of sample required is only 3 ul compared to 10 ul for a 4.6 mm ID of the same length with similar baseline separations.

Instead of a 6 column volume gradient a shallower gradient of 14.5 cv was chosen during a 30 minutes run at 2.4 times the linear flow rates of 870 cm/hr.
15ml of waste was generated during the run as compared with 30 ml for a 4.6 mm ID column with the same length.

Another alternative is the 4mm ID column with the same length for comparison.

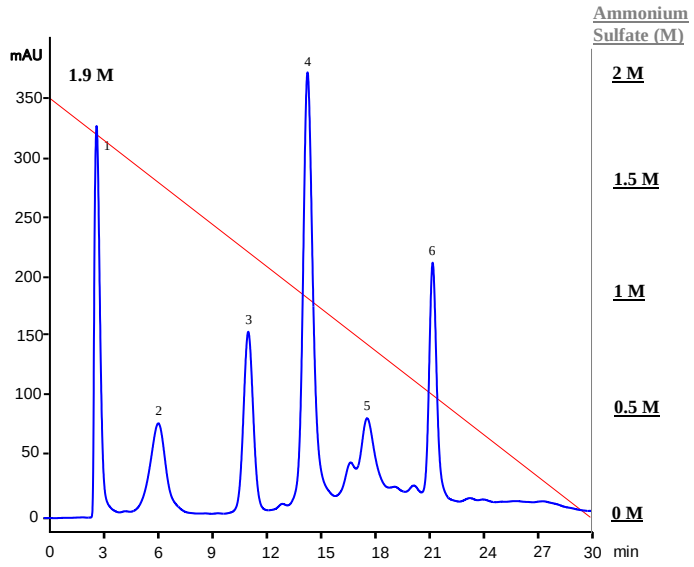

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS™ HIC-Butyl/XH** 4 X 300 mm (v=3.77ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO4(NH4)2, pH=7
Flow rate	1 ml/min (480 cm/hr)
Gradient	90 to 0 % B in 30 min (8 cv)
Temperature	30°C
Detection	214 nm
Injection volume	7 µl
Sample:	1-Cytochrome c, 2-Myoglobin, 3-Ribonuclease A , 4-Lysozyme, 5- Ovalbumin , 6- α-Chymotrypsinogen A 1 mg/ml each in buffer A.

The columns are run at volumetric flow rates of 0.5 and 1ml/min. In one case the linear flow rate is 870 cm/hr (narrow bore column with 2.1 mm ID) whereas the linear flow rate of the other is only 480 cm/hr (column with 4 mm ID).
The difference is clear from the chromatograms and the amount of sample required is also shown in each case.

Inc.