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The Vanguard of Liquid Chromatography.

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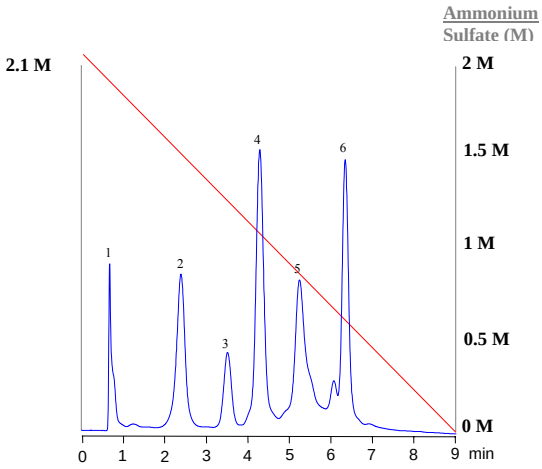
APPLICATION NOTE

Hydrophobic Interaction Chromatography compared with Polymeric Reversed Phase: STYROS™ HIC-Butyl versus STYROS™ 2R.

Hydrophobic Interaction Chromatography or HIC is based on the adsorption of biomolecules such as proteins through non ionic interactions between non polar regions on the protein's surface and the hydrophobic surface of the stationary phase. It is usually performed during an elution starting with high salt concentrations.
The following chromatogram shows the fast separation of 6 proteins on a 10 cm column on HIC mode.



Chromatogram 1
Separation of 5 proteins on STYROS™ HIC-Butyl/XH

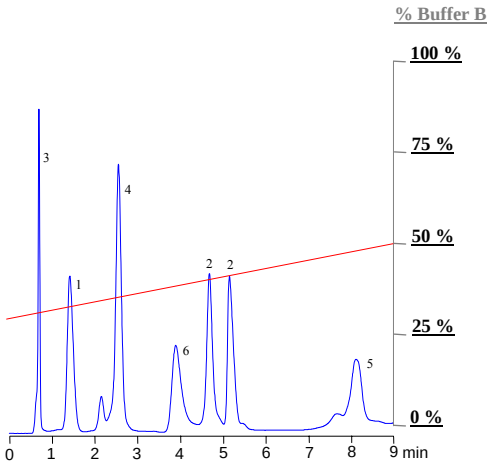
Table 1. Operating parameters for the chromatograms.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HIC-Butyl/XH 4.6 X 100 mm
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO4(NH4)2, pH=7
Flow rate	2 ml/min (720 cm/hr)
Gradient	100 to 0 % B in 9 min (11 cv)
Temperature	30°C
Detection	280 nm
Injection volume	10 µl
Sample:	1-Cytochrome c, 0.5 mg/ml, 2-Myoglobin 2.5 mg/ml, 3-Ribonuclease A 5 mg/ml, 4-Lysozyme 2 mg/ml, 5-Ovalbumin 5 mg/ml, 6- α-Chymotrypsinogen A 2.5 mg/ml in buffer A.

HIC is used following a step that resulted in high salt concentration such as precipitation of the protein with ammonium sulfate, ion exchange chromatography or simply as the initial step from the salt containing biological medium.

Reversed phase chromatography or RPC, on the other hand consists of binding the proteins in a polar mobile phase and reducing the polarity of the mobile phase during elution.

The reversed phase separation of the same mixture of proteins is shown in the following chromatogram with a similar size column.



Chromatogram 1
Separation of 5 proteins on STYROS™ 2R/XH

Table 2. Operating parameters for the chromatograms.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ 2R/XH 4.6 X 100 mm
Mobile phase.	A: 0.075 % TFA in H2O. B: 0.075 % TFA in ACN:H2O (95:5)
Flow rate	2 ml/min (720 cm/hr)
Gradient	30 to 50 % B in 9 min (11 cv)
Temperature	30°C
Detection	280 nm
Injection volume	10 µl
Sample:	1-Cytochrome c, 1 mg/ml, 2-Myoglobin 2.5 mg/ml, 3-Ribonuclease A 1.5 mg/ml, 4-Lysozyme 1 mg/ml, 5-Ovalbumin 5 mg/ml, 6- α-Chymotrypsinogen A 2.5 mg/ml in buffer A.

Comparison of the two methods:

HIC	RPC
Non denatured proteins	*Denatured proteins*
Adsorption chromatography	*Partition chromatography*
Weaker interaction	*Stronger interaction*
Less hydrophobic ligands	*More hydrophobic ligands*
Elution with reducing salt in water.	*Elution with organic non polar solvents.*
Matrix less substituted	*Matrix more substituted*

With STYROS™ columns both methods can be used in high and low pressure chromatography mode.

Inc.