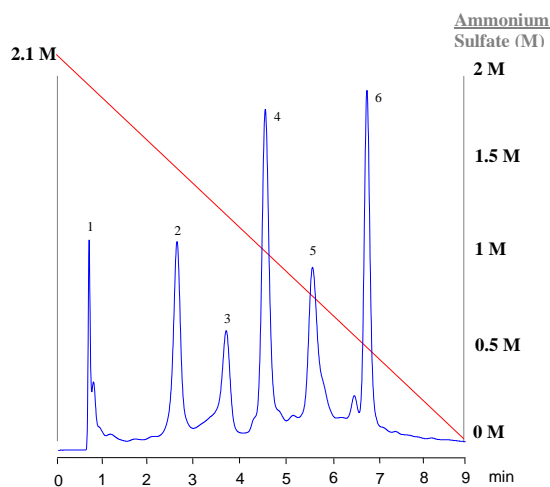


APPLICATION NOTE

Hydrophobic Interaction Chromatography : Comparison of STYROS™ HIC-Phenyl with Shodex HIC PH-814.

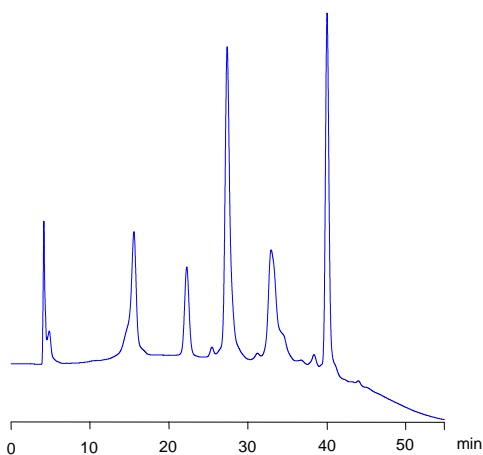
A mixture of 6 proteins were separated on a **STYROS™ HIC-Phenyl** 4.6 x 100 mm column (volume 1.7 ml) at linear flow rates of 720 cm/hr and 120 cm/hr and compared with the performance of a typical Shodex HIC Phenyl column of 8.0 mm x 75 mm (volume 3.8 ml) run at a linear flow rate of 120 cm/hr (1 ml/min volumetric flow).

The results show a clear advantage with the **STYROS™** column both in performance and operating conditions.



Chromatogram 1

Separation of 6 proteins on **STYROS™ HIC-Phenyl/XH** (Linear Flow Rate: 720 cm/hr)

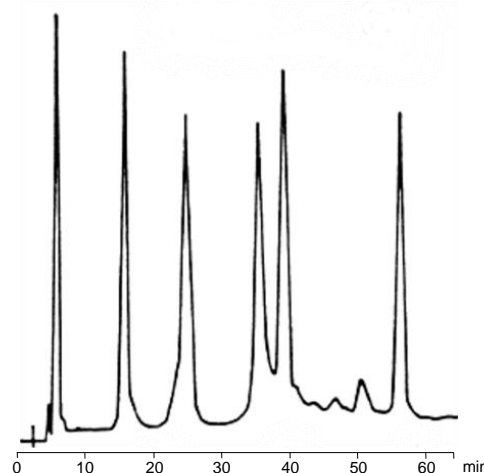


Chromatogram 2

Separation of 6 proteins on **STYROS™ HIC-Phenyl/XH** (Linear Flow Rate: 120 cm/hr)

Table 1. Operating parameters for chromatograms 1 and 2.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HIC-Phenyl/XH 4.6 X 100 mm
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 2.1 M SO ₄ (NH ₄) ₂ , pH=7
Flow rate	2 ml/min (720 cm/hr) and 0.33 ml/min (120 cm/hr)
Gradient	100 to 0 % B in 9 min (11 cv) and 100 to 0% B in 55 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.



Chromatogram 3

Separation of 6 proteins on **Shodex™ HIC PH-814** (Linear Flow Rate: 120 cm/hr)

Table 2. Operating parameters.

Columns	Shodex™ HIC PH-814 (8.0 mm X 75 mm)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 1.8 M SO ₄ (NH ₄) ₂ , pH=7
Flow rate	1 ml/min (120 cm/hr)
Gradient	100 to 0 % B in 60 min (15.9 cv)
Temperature	30°C
Detection	280 nm
Sample:	1-Cytochrome c, 0.03%, 2-Myoglobin 0.08%, 3- Ribonuclease A 0.16%, 4- Ovalbumin 0.16%, 5- Lysozyme 0.04%, 6- α-Chymotrypsinogen 0.05%.

