OraChrom, Inc.
The Vanguard of Liquid Chromatography.

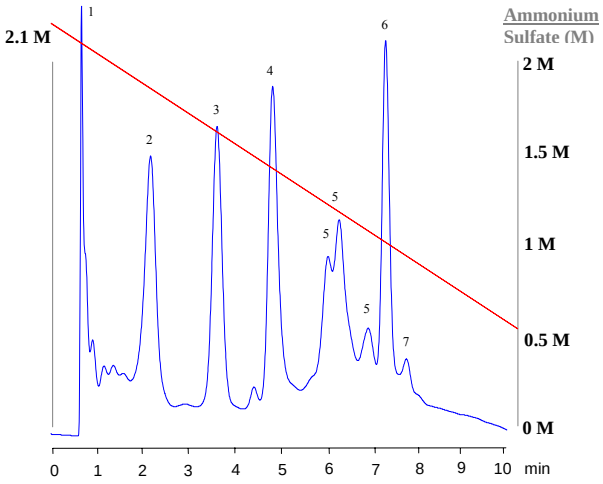
10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: *info@orachrom.com* *orachrom.com*

APPLICATION NOTE

Hydrophobic Interaction Chromatography: Comparison of STYROS™ HIC-Ether with TSKgel Ether 5PW from TOSOH.

A mixture of 7 proteins were separated on a STYROS™ HIC-Ether 4.6 x 100 mm column (volume 1.7 ml) at linear flow rates of 720 cm/hr (2 ml/min volumetric flow) and compared with the performance of a TOSOH TSKgel Ether-5PW 7.5 mm x 7.5 cm, column (volume 3.3 ml) run by the manufacturer at a linear flow rate of 136 cm/hr (1 ml/min volumetric flow) using 5 proteins.

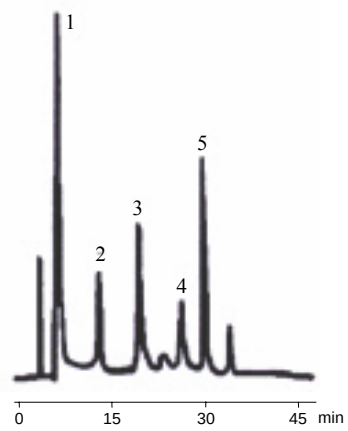
Both resins are porous; STYROS™ is a Simulated Monolith™ column made of hard gel polymeric that can withstand high back pressures and therefore can be run at high flow rates, whereas TSKgel is a soft gel resin with pressure and flow rates limitations.



Chromatogram 1
Separation of 7 proteins on STYROS™ HIC-Ether/XH
(Linear Flow Rate: 720 cm/hr)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ HIC-Ether/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 30 % B in 10 min. (12.5 cv)
Temperature	30°C
Detection	280 nm
Injection volume	10 µl
Sample:	1- Cytochrome c, 0.1 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-Chymotrypsin 2.5 mg/ml, 7- α-Chymotrypsinogen A 0.5 mg/ml in buffer A.



Chromatogram 2
Separation of 5 proteins on TSKgel Ether-5PW
(Linear Flow Rate: 136 cm/hr)

Table 2. Operating parameters.

Columns	TSKgel Ether-5PW, (7.5 cm X 7.5 mm)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 1.8 M SO4(NH4)2, pH=7
Flow rate	1 ml/min (136 cm/hr)
Gradient	100 to 0 % B in 60 min (18 cv)
Temperature	25°C
Detection	280 nm
Injection volume	100 µl (50-100 µg)
Sample	1-Myoglobin, 2-Ribonuclease, 3-Lysozyme, 4-α-Chymotrypsin, 5-α-Chymotrypsinogen.

TSKgel Ether-5PW is a soft gel that operates at low back pressure (150 psi), with the maximum pressure being 300 psi.

STYROS™ media in comparison are made of fully pervious polymeric hard gel. They can withstand back pressures of up to 3000 psi.

The back pressure of the 4.6x100mm column in the present application run at 2 ml/min is under 1500 psi at the start of the gradient with 2.1 M salt.

It is therefore possible to run separations at low flow rates with low back pressures for FPLC applications and also use longer STYROS™ columns (250mm) to explore added resolution.

Such versatility is very unique with this line of products.

Inc.