OraChrom, Inc.
The Vanguard of Liquid Chromatography.

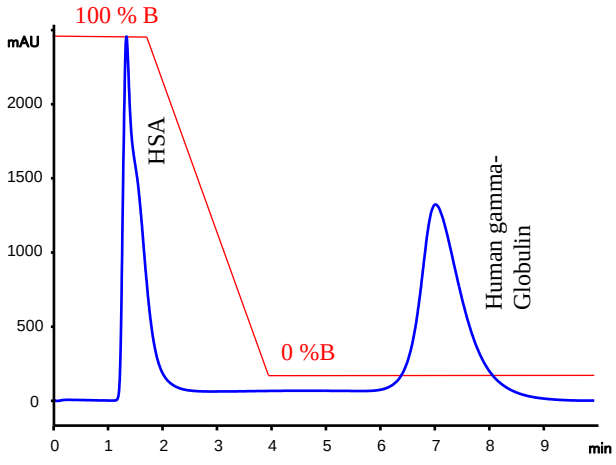
10-B Henshaw Street Woburn, MA 01801 USA Phone (781) 932 0151 Fax (781) 932 0787
E-mail: *info@orachrom.com* *www.orachrom.com*

APPLICATION NOTE

Hydrophobic Interaction Chromatography: Separation on Narrow Bore Columns of gamma-Globulin from Serum Albumin (bovine and human blood).

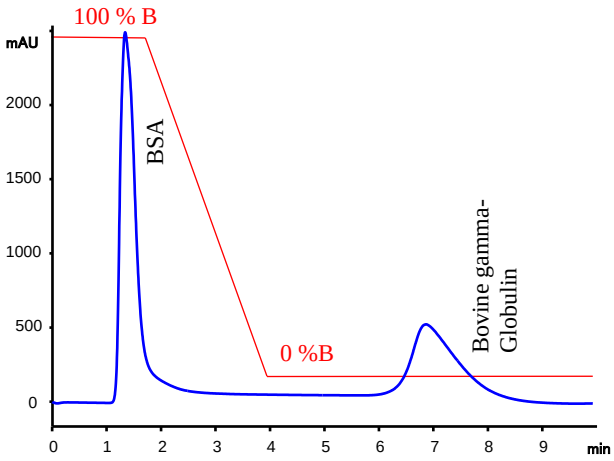
The ever-increasing sensitivity of detectors including the hyphenation with mass spectrometers as a powerful means of detection, the field of chromatography has shifted its focus from high resolution separations of the media to a higher compatibility with mass spectrometers.
The use of Narrow Bore, microbore and capillary columns is also an important feature of new detection modes.
It not only addresses the environmental concerns of waste generation and disposal, it is also cognizant of the limited supply of samples primarily during the initial phases of a project.

This application note highlights the capability of STYOS® HIC to run high salt separations of proteins at high flow rates and low back pressures.
Made of polystyrene cross-linked with divinylbenzene they offer the stability of hard gel media with high dynamic capacities.
Their Simulated-Monolith™ feature makes the pore size requirements obsolete.
They readily lend themselves to continuous processing such as Simulated Moving Bed, enhancing the capabilities of large productions without the encumbrances of soft gel.
More importantly their use results in drastic reduction of the manufacturing facility footprint making it possible to respond to the need for any accelerated production in a short period of time and at large scales.
In the present application we have used STYROS® HIC-Butyl to separate Bovine and human Serum Albumin from its gamma-globulin.
The pressure drop on the system at 0.4 ml/min, is less than 30 bars at the start of the gradient with 1.2 M Ammonium Sulfate and decreases during the gradient as the salt concentration decreases.



Chromatogram 1
STYROS® HIC-Butyl/NB Simulated-Monolith™
Flow Rate: 0.4 ml/min.

The column is run at volumetric flow rates of 0.4 ml/min. That is a linear velocity of 700 cm/hr. for an empty column, compared to 130 cm/hr for soft gel media.



Chromatogram 2
STYROS® HIC-Butyl/NB Simulated-Monolith™
Flow Rate: 0.4 ml/min.

Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	**STYROS® HIC-Butyl/XH** 2.1 X 150 mm (v=0.52 ml)
Mobile phase.	A: 0.1 M Phosphate, pH=7 B: A + 1.2 M SO4(NH4)2, pH=7
Flow rate	0.4 ml/min (700 cm/hr. on an empty column)
Gradient	100 % B for 2 minutes, to 0% B in 4 minutes, and 0% B to 10 minutes.
Temperature	30°C
Detection	214 nm
Injection volumes	5 µl
Sample:	5 mg/ml of each component in buffer A.

The linear flow rate can be increased to 1 ml/min without major increase in pressure drop as well as performance.
The same separation is done on a reversed phase STYROS® 1R in Application Note 131.

Inc.