OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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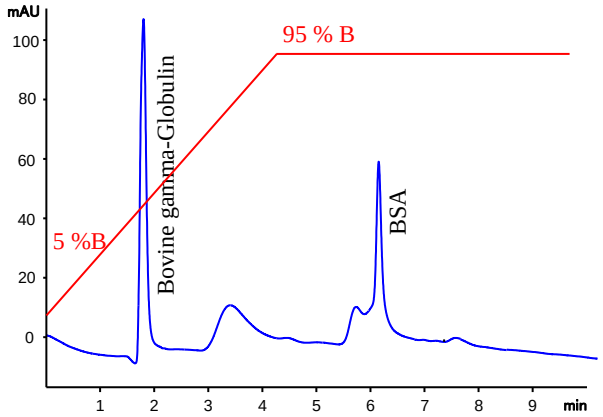
APPLICATION NOTE

Reversed Phase Separations of gamma-Globulin from Serum Albumin (bovine and human blood) using Narrow Bore Columns and MeOH.

To avoid the use of salt during hyphenation with mass spectra, one has the option of reversed phase chromatography instead of HIC.
Although in both HIC and Reversed Phase chromatography hydrophobicity of samples are at play during separations, the exposure of the hydrophobic sites of proteins differs in these processes for albumin and gamma globulin.
The present application note is a comparison of reversed phase chromatography of albumin with gamma globulin from human and bovine.
As mentioned previously 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® R to run small amounts of proteins at flow rates above soft gel and the use of narrow bore columns.
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, opening the capabilities of large columns productions with all the Monolithic properties.
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® 1R to separate Bovine and human Serum Albumin from its gamma-globulin.
The pressure drop on the system at 0.2 ml/min, is less than 10 bars at the start of the gradient with 5% buffer B and decreases further with the increase of organic solvent.

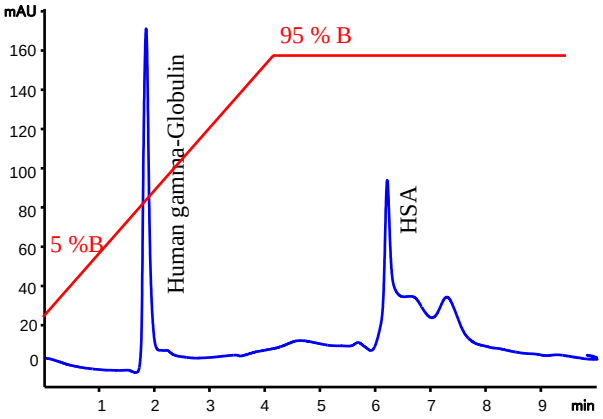


Chromatogram 1
STYROS® 1R/NB Simulated-Monolith™
Flow Rate: 0.2 ml/min.

The column is run at volumetric flow rates of 0.2 ml/min. That is a linear velocity of 350 cm/hr. for an empty column, compared to 130 cm/hr. for soft gel.

Operating conditions.

HPLC System.	Agilent 1290 Infinity with thermostatted column compartment.
Columns	**STYROS® 1R/NB 2.1X 150 mm**
Mobile phase.	A: 5% MeOH in DI H2O B: 100% MeOH
Flow rate	0.2 ml/min, 350 cm/hr. in an empty column.
Gradient	5 to 95 % B in 5 minutes
Temperature	37°C
Detection	214 nm
Injection volume	2 µl
Pressure Drop	Less than 10 bars at the start of the gradient.
Sample:	5 mg/ml of each component in buffer A.



Chromatogram 2

STYROS® 1R/NB Simulated-Monolith™
Flow Rate: 0.2 ml/min.

The linear flow rate can be increased to 1 ml/min without major increase in pressure drop as well as performance.
The elution of the proteins has shifted, and additional variances are revealed when operating on reversed phase compared to HIC in Application Note 130

Inc.