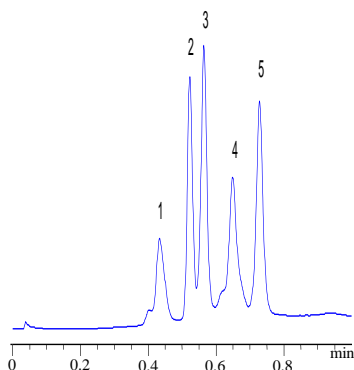


## APPLICATION NOTE

### Ultra Fast Separation of Protein on Narrow Bore Columns.

Considering the significance of time and sample consumption during method development, one can understand the importance of smaller volume columns such as narrow bore in trying different combination of eluent as well as gradient profiles in the shortest possible time with minimal use of high value sample. It is possible to separate a complex mixture of proteins in less than one minute using a 2.1x50 mm column.



**Chromatogram 1:** Separation of standard protein mixture at 5 ml/min (8,600 cm/hr) on a 50x2.1mm **STYROS™ 1 R/NB** column. Sample volume: 0.2 µl.

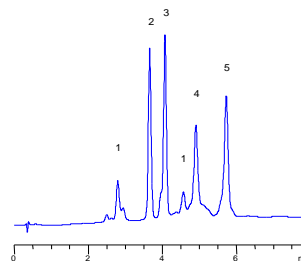
The general chromatographic conditions of the different chromatograms are summarized in the following table.

**Table 1. Operating Parameters for Chromatograms 1-5.**

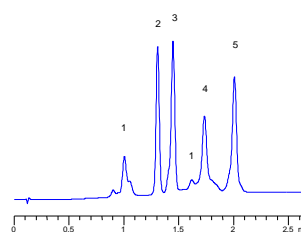
<b>HPLC System.</b>	HP 1100
<b>Column</b>	<b>STYROS™ 1 R/NB</b> 50x2.1; 100x2.1 and 250x2.1mm
<b>Mobile Phase</b>	A: 0.1% (v/v) TFA in water B: 0.1% (v/v) TFA in Acetonitrile/water 95/5 (v/v)
<b>Flow rate</b>	0.5-5 ml/min
<b>Gradient</b>	15% B to 80% B in 25 Column Volume
<b>Temperature</b>	Ambient
<b>Detection</b>	214 nm
<b>Injection volume</b>	0.2 µl on 5 and 10 cm long columns 1.0 µl on 25 cm long column
<b>Sample 1 mg/ml each</b>	1: Soybean Trypsin Inhibitor, 2: Cytochrome C, 3: Lysozyme, 4: Hemoglobin, 5: Ovalbumin

The ideal stationary phase for this type of application is one that has minimal or no change in resolution at high flow rates.

The set of separations performed on a 10 cm narrow bore column shows the performance of **STYROS™ 1 R/NB** at flow rates ranging from 1,700 cm/hr to 5,100 cm/hr. The resolution remains the same.

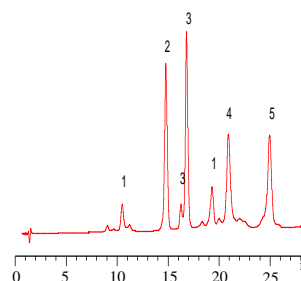


**Chromatogram 2:** Separation of standard protein mixture at 1ml/min (1,700 cm/hr) on a 100x2.1 mm **STYROS™ 1 R/NB** column. Sample volume: 0.2 µl.



**Chromatogram 4:** Separation of standard protein mixture at 3 ml/min (5,100 cm/hr) on a 100x2.1 mm **STYROS™ 1 R/NB** column. Sample volume: 0.2 µl.

The 250x2.1 mm column offers a step higher resolution, still within the same narrow bore domain, and fast flow.



**Chromatogram 5:** Separation of standard protein mixture at 0.5 ml/min (850 cm/hr) on **STYROS 1 R/NB** 250x2.1 mm column. Sample volume: 1.0 µl.

The present set of Narrow Bore columns provides a potent tool to the research community in their quest to reach their goals in the shortest, most manageable way.