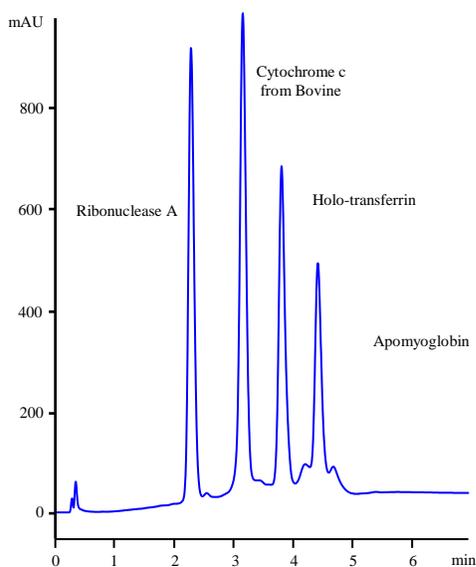


## APPLICATION NOTE

### STYROS™ 3R Simulated Monolith™ Polymeric Reversed Phase. Protein Standard Separation at High Linear Velocity.

Hard gel polymeric gigaporous stationary phases offer similar mechanical stability compared to silica with far superior capacity and chemical stability regarding extremes of pH's.

The present application note highlights the capabilities of **STYROS™ 3R Simulated Monolith™** in the separation of biomolecules at high linear velocity.



**Chromatogram 1**

Separation of Standard proteins on **STYROS™ 3R/XH**  
Flow Rate: 2.5 ml/min (900 cm/hr)

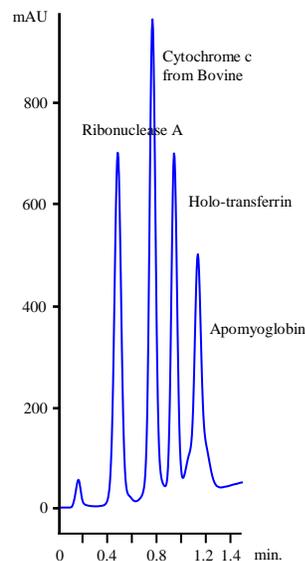
**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ 3R/XH</b> 4.6 X 50 mm
<b>Mobile phase.</b>	A: 0.1% TFA in H <sub>2</sub> O B: 0.1% TFA in ACN:H <sub>2</sub> O 95:5
<b>Flow rate</b>	2.5 ml/min (900 cm/hr of linear flow rate)
<b>Gradient</b>	15 to 50% B in 5 min. (15 cv)
<b>Temperature</b>	30°C
<b>Detection</b>	220 nm
<b>Injection volume</b>	25 µl
<b>Pressure Drop</b>	12 bar (174 psi)
<b>Sample:</b>	Protein Standard from Sigma: Ribonuclease A, Cytochrome c from bovine, Holo-transferrin, Apomyoglobin, (1 mg/ml each in buffer A)

The same separation can be run at twice the present flow rate and still maintain the baseline.

To this end the gradient is shifted to slightly higher organic and slightly sharper slope of the gradient from 15 to 9 column volumes.

The 4 standard proteins are separated in under 2 minutes. The chromatogram shows a similar sensitivity as the previous run.



**Chromatogram 2**

Separation of Standard proteins on **STYROS™ 3R/XH**  
Flow Rate: 5 ml/min (1,800 cm/hr)

**Table 2. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ 3R/XH</b> 4.6 X 50 mm
<b>Mobile phase.</b>	A: 0.1% TFA in H <sub>2</sub> O B: 0.1% TFA in ACN:H <sub>2</sub> O 95:5
<b>Flow rate</b>	5 ml/min (1,800 cm/hr of linear flow rate)
<b>Gradient</b>	24 to 60% B in 1.5 min. (9 cv)
<b>Temperature</b>	30°C
<b>Detection</b>	220 nm
<b>Injection volume</b>	25 µl (350 psi)
<b>Pressure Drop</b>	24 bar
<b>Sample:</b>	Protein Standard from Sigma: Ribonuclease A, Cytochrome c from bovine, Holo-transferrin, Apomyoglobin, (1 mg/ml each in buffer A)

It is important to keep in mind that the availability of **STYROS™** columns is not limited to the presently featured 4.6 x 50 mm of this application note. Users are encouraged to seek the many alternative columns that enable greater resolutions without compromising the use due to high back pressures in all stages of a separation process.

