

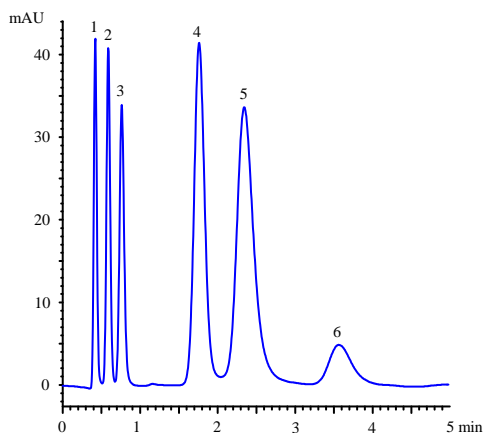
## APPLICATION NOTE

### **STYROS™ 3R Simulated Monolith Polymeric Reversed Phase: Standard Separation of 6 Small Molecules.**

Reversed phase chromatography with silica provides high resolving power but very limited stability due to the solubility of the matrix. The separations are therefore limited to neutral pH's for a limited number of runs.

Polymeric on the other hand have been used successfully to alleviate the issues of chemical instability associated with silica matrices and therefore have provided the possibility of using the full pH range for baseline separations.

The following chromatogram shows the separation of 6 small molecules on a **3R Simulated Monolith** reversed phase column.



**Chromatogram 1**

Separation of 6 small molecules on **STYROS™ 3R/XH**  
 (Flow Rate: 2 ml/min on normal bore column at 30° C)

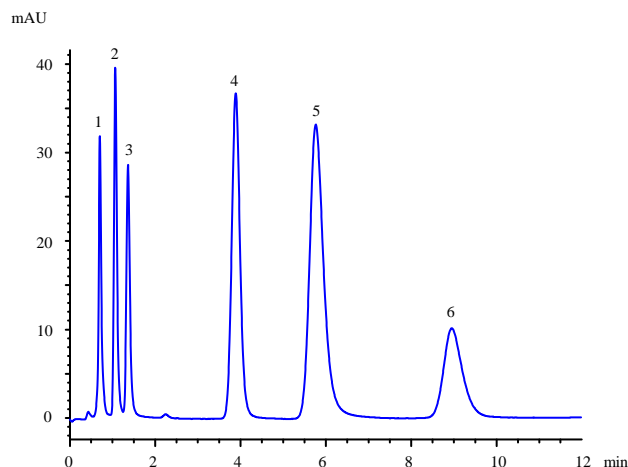
**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ 3R/XH</b> 4.6 X 50 mm (V=0.83 ml)
<b>Mobile phase.</b>	A: H2O B: ACN
<b>Flow rate</b>	2 ml/min (720 cm/hr of linear flow rate)
<b>Gradient</b>	Isocratic: 40 % B
<b>Temperature</b>	30°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	10 µl
<b>Sample:</b>	1. Thiourea, 2. Benzyl alcohol, 3. Aniline, 4. Nitrobenzene, 5. Methyl Salicylate, 6. Toluene (30-300 ug/ml each in A:B 50:50)

Full separation can be achieved on a short 5 cm column at linear flow rates of 700 cm/hr.

Increasing the column length to 10 cm and raising the temperature to 60° C the separation can be improved further even on a narrow bore column of 2.1mm ID and linear flow rates of 1,200 cm/hr.

Symmetrical peaks indicate the absence of surface charge.



**Chromatogram 2**

Separation of 6 small molecules on **STYROS™ 3R/NB**  
 (Flow Rate: 0.7 ml/min on narrow bore column)

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1100 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS™ 3R/NB</b> 2.1 X 100 mm (V=0.35 ml)
<b>Mobile phase.</b>	A: H2O B: ACN
<b>Flow rate</b>	0.7 ml/min (1,200 cm/hr of linear flow rate)
<b>Gradient</b>	Isocratic: 25 % B
<b>Temperature</b>	60°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	2.5 µl
<b>Sample:</b>	1. Thiourea, 2. Benzyl alcohol, 3. Aniline, 4. Nitrobenzene, 5. Methyl Salicylate, 6. Toluene (30-300 ug/ml each in A:B 50:50)

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

The present application note highlights the capabilities of **STYROS™ 3R**.

Unlike Monolith **STYROS™ Simulated Monolith** columns are available in many sizes for additional resolving capabilities.

