

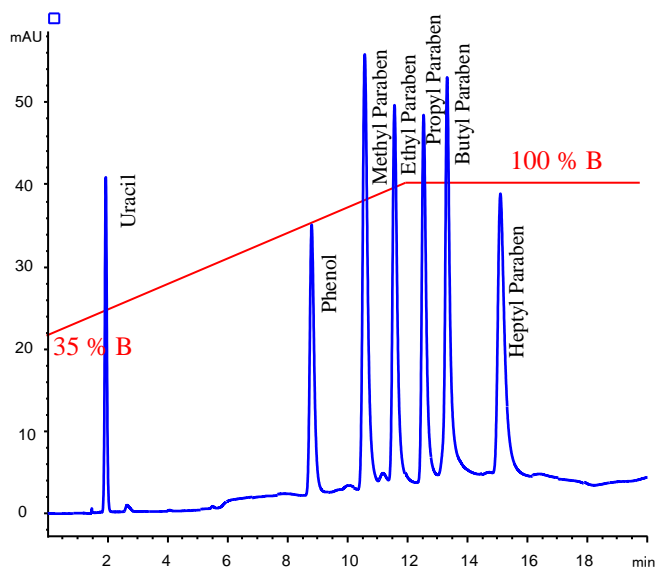
## APPLICATION NOTE

### STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase.

#### Advantages of using Narrow Bore instead of Normal Bore columns for LC separations.

The improvement of mass spectrometers has reached a point where the injection of a mixture allows the detection of its components without the need of any prior separation on an LC column. The focus is now the contamination of the samples due to the leaching of the LC columns.

In the present application, we are using a Narrow Bore column of 2.1 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace Normal Bore columns of 4.6 mm ID.



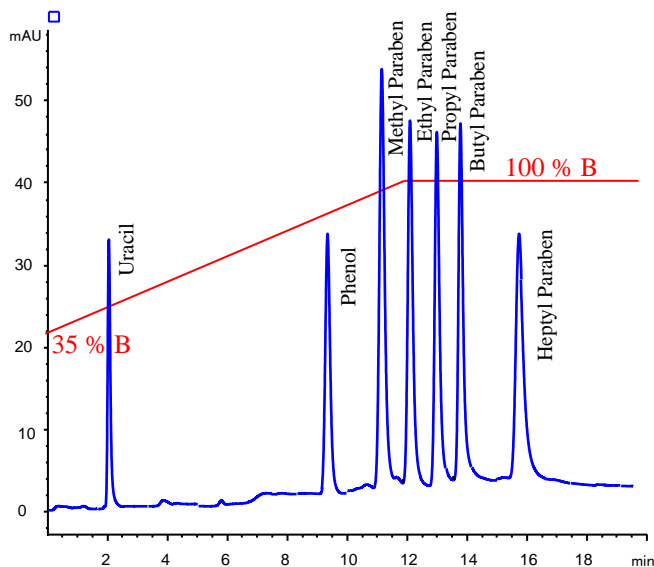
**Chromatogram 1**

Separation of 7 components on **STYROS® 2R/XH** 4.6 x 150 mm  
Flow Rate: 1 ml/min.

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1290 Infinity with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/XH</b> 4.6X150 mm
<b>Mobile phase.</b>	A: DI H <sub>2</sub> O B: MeOH
<b>Flow rate</b>	1 ml/min.
<b>Gradient</b>	35 to 100 % B in 12 minutes at 1ml/min.
<b>Temperature</b>	30°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	10 µl
<b>Pressure Drop</b>	199 bar at the start of gradient (includes the system pressure)
<b>Sample:</b>	Supelco HPLC Gradient System Mix part number 48271

This run is compared with a run using a Narrow Bore column of 2.1 mm ID and the same length. The total solvent used for the run was 12 ml compared to 2.4 ml with the Narrow Bore. 5 times less sample was used as well to generate less waste. The column requires regeneration which does contribute to unnecessary waste generation as well.



**Chromatogram 2**

Separation of 7 components on **STYROS® 2R/NB** 2.1x150 mm  
Flow Rate: 0.2 ml/min.

**Table 2. Operating parameters.**

<b>HPLC System.</b>	Agilent 1290 Infinity with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/NB</b> 2.1X150 mm
<b>Mobile phase.</b>	A: DI H <sub>2</sub> O B: MeOH
<b>Flow rate</b>	0.2 ml/min.
<b>Gradient</b>	35 to 100 % B in 12 minutes at 0.2 ml/min.
<b>Temperature</b>	30°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	2 µl
<b>Pressure Drop</b>	90 bar at the start of gradient (includes the system pressure)
<b>Sample:</b>	Supelco HPLC Gradient System Mix part number 48271

As Simulated-Monolith™ the separations can be run at high linear velocities and so can the column regeneration. This is now an example of small molecules being readily separated with Narrow Bore columns. It is important to keep in mind the dwell volume of the instrument when using small bore columns as too large of a dwell volume is not helpful in properly achieving the required gradient.

