

## APPLICATION NOTE

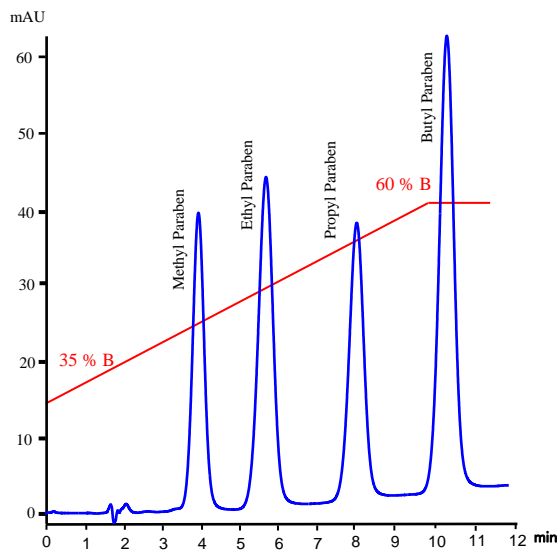
### STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase.

#### Separation of 4 parabens on Capillary column of 0.5 mm ID. Comparison with Micro Bore of 1 mm ID.

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 as a result of leaching of the LC columns.

In the present application we are using a Capillary column of 0.5 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace Micro Bore columns of 1 mm ID.



**Chromatogram 1**

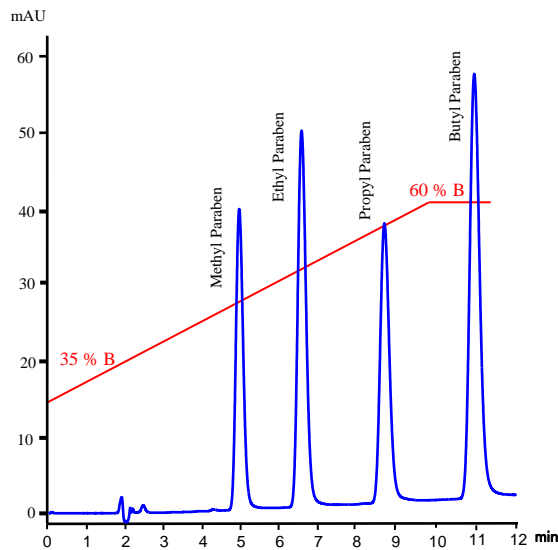
Separation of 4 Parabens on **STYROS® 2R/Cap**  
Flow Rate: 0.04 ml/min.

**Table 1. Operating parameters.**

<b>HPLC System.</b>	Agilent 1290 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/Cap 0.5 X 300 mm</b>
<b>Mobile phase.</b>	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
<b>Flow rate</b>	0.04 ml/min.
<b>Gradient</b>	35 to 60 % B in 10 minutes (~ 6 cv)
<b>Temperature</b>	60°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	0.5 µl
<b>Pressure Drop</b>	150 bar (~ 2100psi) at the start of gradient
<b>Sample:</b>	4 Parabens from Sigma: as indicated on the chromatogram

The media does not leach and can be used with mass spectrometer. The size of the column allows minimal splitting to the waste for the hyphenation.

Compared with the Micro Bore column, 50 % less of eluent and sample are needed for the separation.



**Chromatogram 2**

Separation of 4 Parabens on **STYROS® 2R/MB**  
Flow Rate: 0.1 ml/min.

**Table 2. Operating parameters.**

<b>HPLC System.</b>	Agilent 1290 with thermostatted column compartment.
<b>Columns</b>	<b>STYROS® 2R/MB 1 X 300 mm</b>
<b>Mobile phase.</b>	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
<b>Flow rate</b>	0.1 ml/min.
<b>Gradient</b>	35 to 60 % B in 10 minutes (~6 cv)
<b>Temperature</b>	60°C
<b>Detection</b>	214 nm
<b>Injection volume</b>	1 µl
<b>Pressure Drop</b>	124 bar (~1800 psi)
<b>Sample:</b>	4 Parabens from Sigma: as indicated on the chromatogram

As Simulated-Monolith™ the separations can be run at high linear velocities as noted above so can the column regeneration.

This is now an example of small molecules being readily separated with Micro Bore as well as Capillary 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.

