

## APPLICATION NOTE

### Polymeric Reversed Phase compared with Mixed-Mode Simulated-Monolith™: STYROS® 1R versus STYROS® HQ-C8.

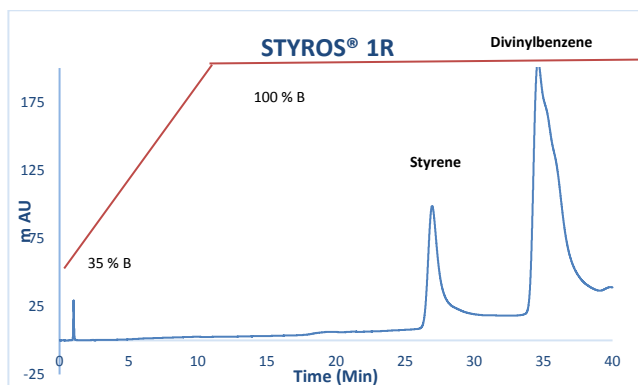
*Mixed-Mode Chromatography or MMC also consists of using a hydrophobic alkyl chain on a quaternary amine anion.*

A mixture of Styrene and Divinyl benzene were run on a reversed phase polymeric STYROS® column.

Despite the aggressive gradient, the separation is far from being conclusive.

The  $\pi$ - $\pi$  interactions of the reversed phase column are too strong for the hydrophobicity of these compounds.

Such tests are run by manufacturers on GC columns giving them an estimation of the content of the products.



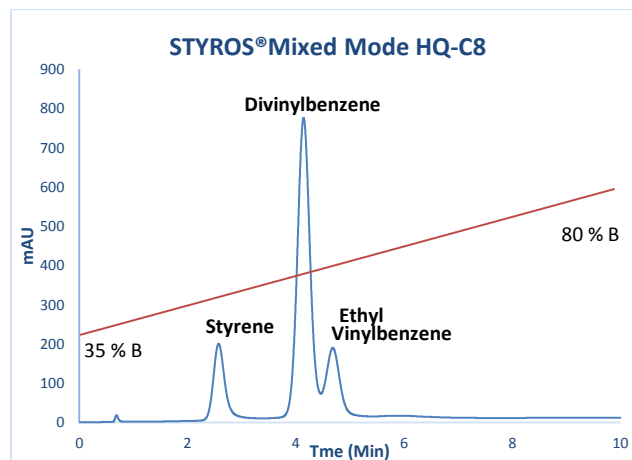
**Chromatogram 1**

<b>HPLC System.</b>	Acquity UPLC / CLASS PLUS
<b>Columns</b>	STYROS® 1R/NB Simulated-Monolith™ 2.1x50 mm
<b>Mobile Phase For reversed phase.</b>	A: 2% ACN in H2O with 0.075% TFA B: ACN: DI H2O (70:30) with 0.075% TFA
<b>Flow rate</b>	0.2 ml/min. Back pressure 620 psi
<b>Gradient</b>	35 to 100 % B in 10 minutes, 100 % B to 40 minutes.
<b>Temperature</b>	37°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	0.5µl
<b>Sample:</b>	Styrene + Divinylbenzene 5 mg/ml each in buffer B.

The same mixture can be run on a Mixed Mode phase to provide a fast and clear separation as shown on the next chromatogram.

In less than 5 minutes, all three compounds in the mixture are eluted from the column revealing the presence of ethyl vinylbenzene.

The separation has room for improvement to have baseline separation of all components of the mixture.



**Chromatogram 2**

<b>HPLC System.</b>	Acquity UPLC / CLASS PLUS
<b>Columns</b>	STYROS® HQ-C8/NB Simulated-Monolith™ 2.1x50 mm
<b>Mobile Phase For reversed phase.</b>	A: 2% ACN in H2O with 0.075% TFA B: ACN: DI H2O (70:30) with 0.075% TFA
<b>Flow rate</b>	0.2 ml/min. Back pressure 525 psi (System included)
<b>Gradient</b>	35 to 80 % B in 10 minutes.
<b>Temperature</b>	37°C
<b>Detection</b>	254 nm
<b>Injection volume</b>	0.5µl
<b>Sample:</b>	Styrene + Divinylbenzene 5 mg/ml each in buffer B

This application Note highlights the higher limits of the MMC and its usefulness in use as multimodal chromatography with broader use.

The Mixed Mode or MMC operates on a hydrophilic base of quaternary amine to which a hydrophobic tail operating on the van der Waals forces is attached.

Whereas the PS-DVB operates solely on aryl entities and  $\pi$ - $\pi$  interactions.

This mode can also be operated with a reversed phase regimen. The softer mixed mode phase, also polymeric, however milder hydrophobic tentacles, readily elutes and separates them.

