

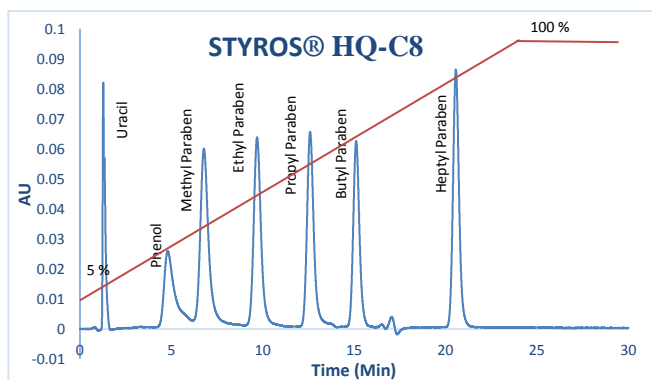
APPLICATION NOTE

Mixed-Mode Stationary Phase compared with Polymeric Reversed Phase: STYROS™ HQ-C8 versus STYROS™ 1R.

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

This mode can also be operated with a reversed phase regimen. To highlight its property, we have chosen a standard sample of 7 compounds used to assess media with a gradient method.

The following chromatogram shows the baseline separation on a 10 cm column.



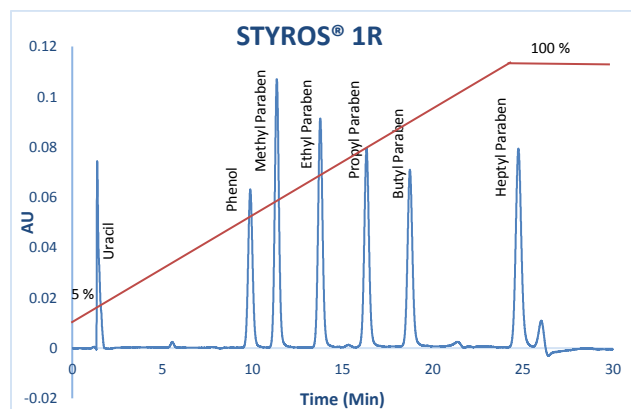
Chromatogram 1

Separation of 7 compounds on **STYROS® HQ-C8**

HPLC System.	Acquity UPLC / CLASS PLUS
Columns	STYROS® HQ-C8 Simulated-Monolith™ 2.1x100 mm
Mobile Phase For reversed phase.	A: 2% ACN in H2O with 0.075% TFA B: ACN: DI H2O (70:30) with 0.075% TFA
Flow rate	0.2 ml/min. Back pressure 1,337 psi (System included)
Gradient	5 to 100 % B in 25 minutes, 100 % B to 30 minutes.
Temperature	37°C
Detection	254 nm
Injection volume	6 µl
Sample:	Certified reference sample (Sigma 48271) with 7 compounds.

The reversed phase separation of the same standard mixture shows a similar elution, however except for Uracil, the other compounds are more retained.

The two columns are the same size, and the same gradient is operated with a similar volumetric flow rate of 0.2 ml/min.



Chromatogram 2

Separation of 7 compounds on **STYROS® 1R**

HPLC System.	Acquity UPLC / CLASS PLUS
Columns	STYROS® 1R Simulated-Monolith™ 2.1x100 mm
Mobile Phase For reversed phase.	A: 2% ACN in H2O with 0.075% TFA B: ACN: DI H2O (70:30) with 0.075% TFA
Flow rate	0.2 ml/min. Back pressure 1,260 psi
Gradient	5 to 100 % B in 25 minutes, 100 % B to 30 minutes.
Temperature	37°C
Detection	254 nm
Injection volume	6 µl
Sample:	Certified reference sample (Sigma 48271) with 7 compounds.

The clear difference between the two stationary phase is the operating mode.

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 π - π interactions.

The clear differences between the two phases in reversed phase mode using small molecules can be seen.

In the next Application Note larger molecules are explored. The advantages of MMC, only with the use of reversed phase would become more apparent.

