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APPLICATION NOTE

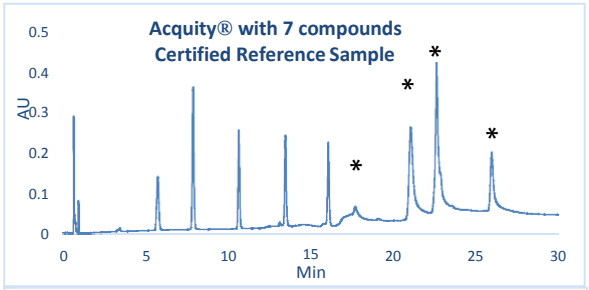
Assessing Narrow Bore Reversed Phase Columns.

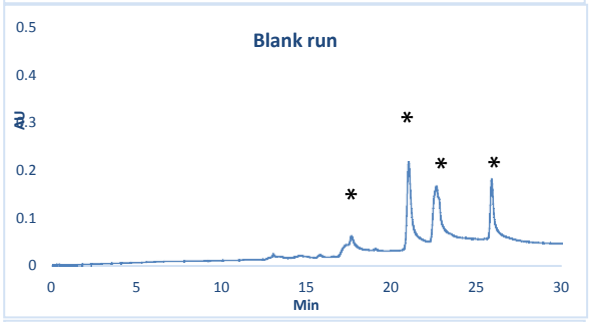
Assessing columns is essential to understand their range of use prior to entertaining any research.

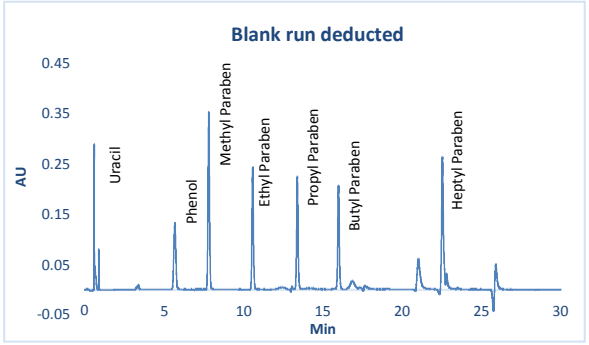
This application note compares three reversed phase columns with a certified reference sample containing 7 compounds (Sigma 48271) applying the same gradient and utilizing a Waters Acquity UPLC *I* CLASS PLUS as well as an Agilent 1290 Binary, both with PDA Detectors. The Narrow bore (NB) columns (2.1 x 50 mm) were selected for their efficient purification, increase in sensitivity, and lower solvent consumption for environmental concerns.

The first column is a Waters Acquity UPLC® HHS C18 1.8 µm 2.1x50 mm (Part No. 186003532)

HPLC System.	Acquity UPLC *I* CLASS PLUS
Columns	Waters Acquity UPLC® HHS C18 1.8 µm 2.1x50 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 2,600 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.

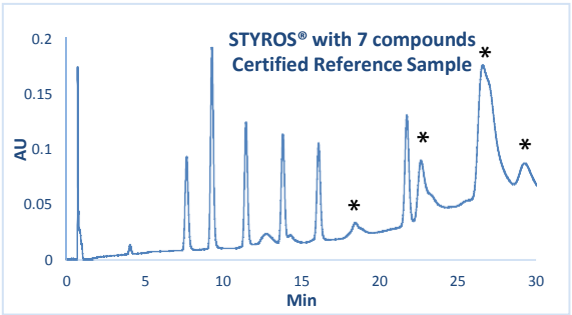


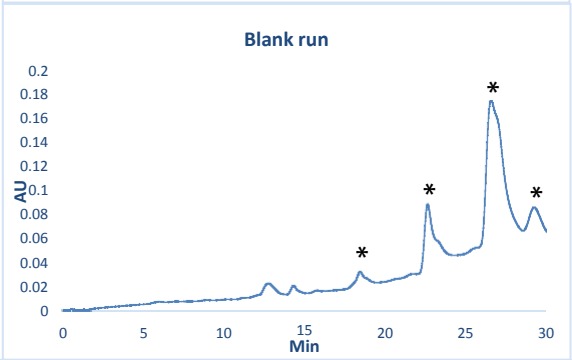


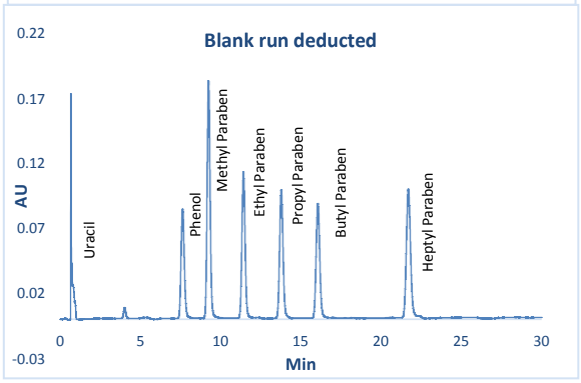


The second column is a STYROS® 1R Simulated Monolith™ 2.1 x 50 mm (Part No. 01-1043-01).

HPLC System.	Acquity UPLC *I* CLASS PLUS
Columns	STYROS® 1R Simulated-Monolith™ 2.1x50 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.



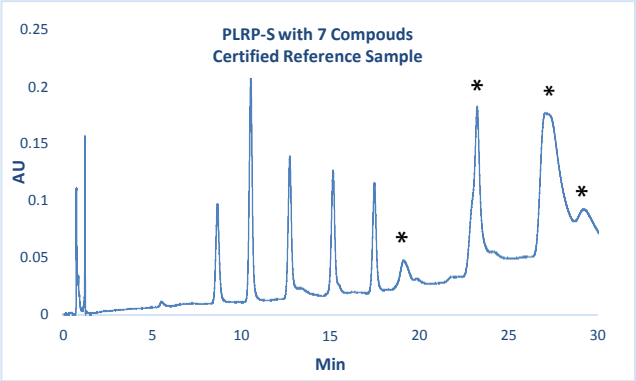


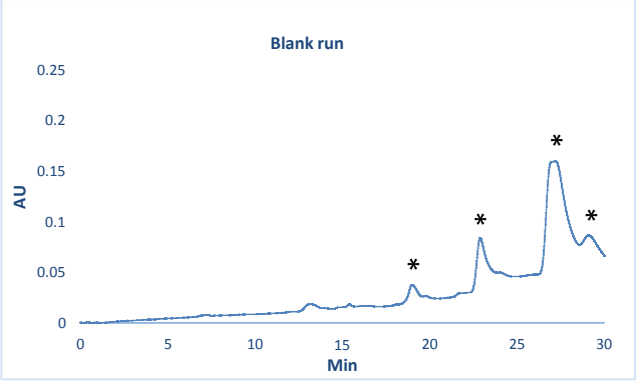


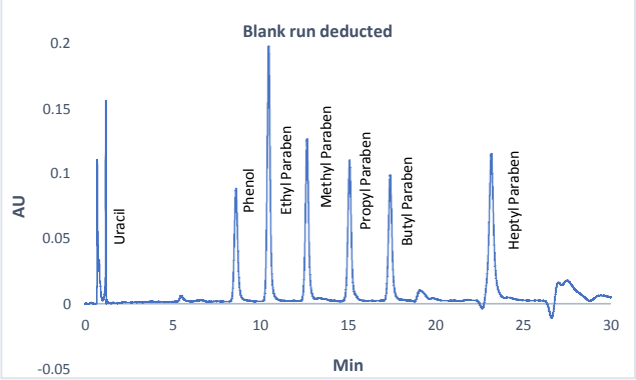
Comparing the C18 column to STYROS® 1R shows the same elution sequence for all 7 compounds. The sharper peaks observed with silica C 18 is at the expense of a higher back pressure (i.e., near double the STYROS® 1R). Some questionable peaks show on the chromatogram of the C18 column that needs further investigation as it could be related to materials leaching from the solid phase media.

The third column PLRP-S 100 A° 3µm 2.1 x 50 mm (Part No. PL 1912-1300).

HPLC System.	Acquity UPLC *I* CLASS PLUS
Columns	PLRP-S 100 A° 3µm 2.1 x 50 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 2,015 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.



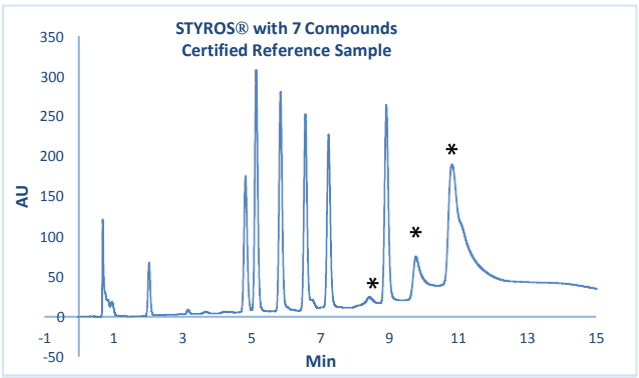


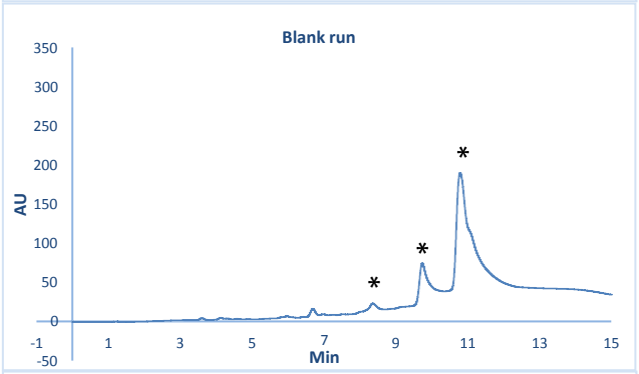


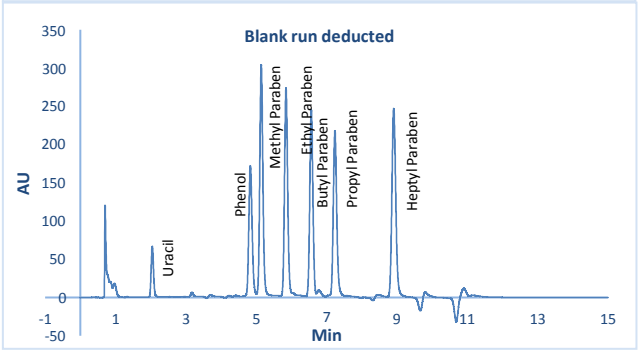
This is now a head-to-head comparison of 2 polymeric columns:
a STYROS® 1R Simulated-Monolith™ with through pores making the size, the shape, and the uniformity of the media obsolete while having a low back pressure whereas the PLRP-S with same NB size and a defined pore size of 100 A° and a bead size of 3 µm displays substantially higher back pressure without resulting in better resolution.
The blank run as well as the peaks raise definite concerns for the leaching of materials from the solid phase media.

The STYROS® Simulated-Monolith™ due to its advantages has been subjected to an additional test by applying a sharper gradient from 70:30 to 95:5 at a faster elution time from 25 to 10 min, which results in chromatic separations at half the time with high resolution (i.e., baseline separation of the 7 compounds).

HPLC System.	Agilent Infinity binary 1290 with PDA
Columns	STYROS® 1R Simulated-Monolith™ 2.1x50 mm
Mobile Phase For reversed phase.	A: DI H2O with 0.075% TFA B: ACN: DI H2O (95:5) with 0.075% TFA
Flow rate	0.2 ml/min. Back pressure 1,200 psi
Gradient	5 to 100 % B in 10 minutes, 100 % B to 15 minutes.
Temperature	37°C
Detection	254 nm
Injection volume	6 µl
Sample:	Certified reference sample (Sigma 48271) with 7 compounds.







Notes: [1] It is a fact that the closer to the limit of pressure the column is, the shorter its lifetime it would be.

* Marks unknown peaks

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