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APPLICATION NOTE

<u>STYROS® 2R Simulated-MonolithTM Polymeric Reversed Phase:</u> Assessment of columns from different manufacturers.

The use of small bore columns is now the norm in most laboratories.

They not only save on solvents but also minimize the use of valuable samples.

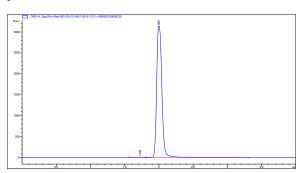
The importance of knowing the column prior to any protocol of validation is crucial in the interpretation of results.

It is therefore highly recommended that columns are assessed at the point of acquisition and monitored periodically for any eventual changes.

Other factors to be considered are the dwell volume of the instrument during gradient elution as well as its performance in isocratic mode.

We have found the Agilent 1290 Infinity to be of superior quality and reliable in performing good separation with small bore or capillary columns.

The followings are chromatograms of STYROS® NB Simulate-Monolith TM polymeric compared with another column of $3\mu m$ particle size with the same dimensions.



Chromatogram 1
Acetone on STYROS® 2R/NB Simulated-MonolithTM
Flow Rate: 0.2 ml/min.

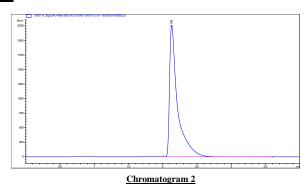
Table 1. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column
	compartment.
Columns	STYROS ® 2R /NB 2.1X 150 mm
Mobile phase.	A: 0.075% TFA in H2O
	B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.2 ml/min
Isocratic	92:8 B:A or 7:1 ACN:H2O
Temperature	30°C
Detection	254 nm
Injection volume	0.5 μl
Pressure Drop	29 bar (~ 420psi)
Sample:	Acetone

The low back pressure of 29 bar is characteristic of STYROS® 2R/NB Simulated-MonolithTM compared to 98 bar for a $3\mu m$ particle size column.

As Simulated-MonolithTM the separations can be run at high linear velocities to allow faster regeneration.

The column can take up to 5,000 psi of pressure.

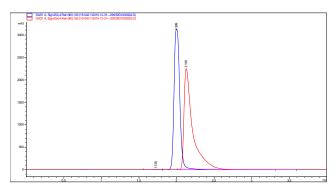


Acetone on same dimension column of 3µm particle size.
Flow Rate: 0.2 ml/min.

Table 2. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column
J J	compartment.
Columns	2.1X 150 mm column with 3 µm particle size
Mobile phase.	A: 0.075% TFA in H2O
	B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.2 ml/min
Isocratic	92:8 B:A or 7:1 ACN:H2O
Temperature	30°C
Detection	254 nm
Injection volume	0.5 μl
Pressure Drop	98 bar (~ 1,420psi)
Sample:	Acetone

Comparison of the performances of the two columns.



Note that as Simulated-Monolith TM the pore size in no longer a factor in separation.

