

APPLICATION NOTE

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

Assessment of columns prior to use.

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.

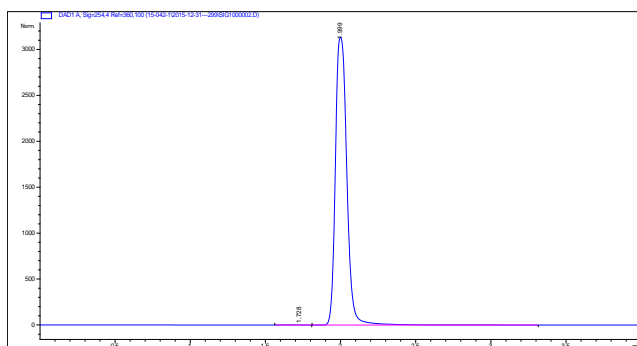
The importance of assessing the column at the start requires a protocol of validation to confidently interpret the results.

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 following are chromatograms of STYROS® NB Simulated-Monolith™ polymeric compared with another column of 3 μm particle size with the same dimensions.



Chromatogram 1

Acetone on **STYROS® 2R/NB** Simulated-Monolith™
Flow Rate: 0.2 ml/min.

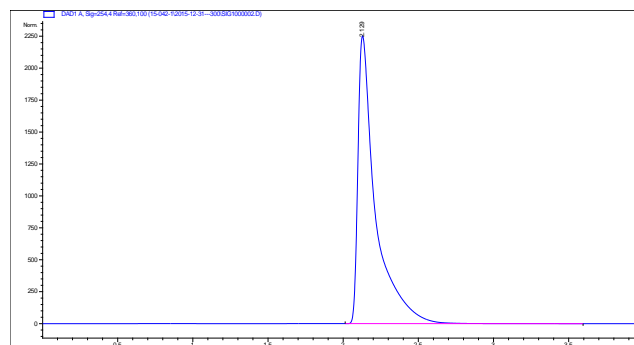
Table 1. Operating parameters

| | |
|-------------------------|--|
| HPLC System. | Agilent 1290 with thermostatted column compartment. |
| Columns | STYROS® 2R/NB 2.1X 150 mm |
| Mobile phase. | A: 0.075% TFA in H ₂ O B: 0.075% TFA in ACN: H ₂ O 95:5 |
| Flow rate | 0.2 ml/min over 9,000 cm/hr. |
| Isocratic | 92:8 B:A or 7:1 ACN:H ₂ O |
| Temperature | 30°C |
| Detection | 254 nm |
| Injection volume | 0.5 μl |
| Pressure Drop | 29bar (~420 psi) |
| Sample: | Acetone |

The low back pressure of 29 bar is characteristic of STYROS® 2R/NB Simulated-Monolith™ compared to 98 bar for a 3 μm particle size column.

As Simulated-Monolith™ the separation can be run at high linear velocities to allow faster regeneration.

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



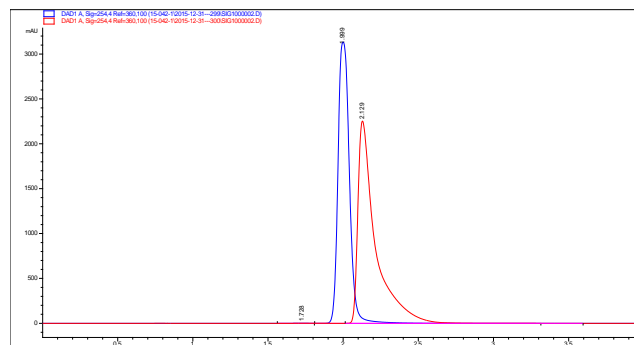
Chromatogram 2

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

Table 2. Operating parameters

| | |
|-------------------------|--|
| HPLC System. | Agilent 1290 with thermostatted column compartment. |
| Columns | 2.1X 150 mm column with 3 μm particle size |
| Mobile phase. | A: 0.075% TFA in H ₂ O B: 0.075% TFA in ACN: H ₂ O 95:5 |
| Flow rate | 0.2 ml/min over 9,000 cm/hr. |
| Isocratic | 92:8 B:A or 7:1 ACN:H ₂ O |
| Temperature | 30°C |
| Detection | 254 nm |
| Injection volume | 0.5 μl |
| Pressure Drop | 98bar (~1,420 psi) |
| Sample: | Acetone |

Comparison of the performances of the two columns under similar conditions and same instrument.



Note that as Simulated-Monolith™ the pore size becomes the controlling factor while particle size becomes obsolete.



Inc.