

APPLICATION NOTE

STYROS® R Simulated-Monolith™ Reversed Phases. Alternative Performances.

Different polymeric reversed phases abound on the market. Those made of polystyrene divinyl benzene with high degree of cross linking are marketed either with superficial pores of 30 to 1000 angstroms (3 to 100 Nanometer) or through pores of 1000 to more than 4000 angstroms (100 to 400 Nanometer).

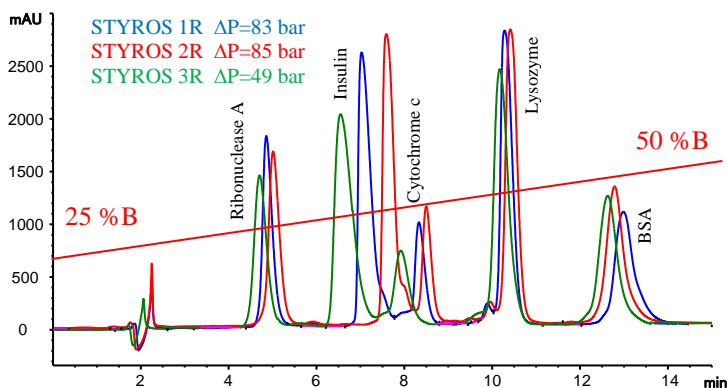
They display different performances and pressure drops depending on the size of the beads.

Monolithic polymers on the other hand have made the dependency of the performance on the size and uniformity of the beads obsolete. The different pore sizes are no longer needed when dealing with molecules of small or large size.

They have however limitations in their production as well as shortcomings such as “wall effects” that has hampered their widespread use in the market.

Simulated-Monoliths™ STYROS® from **OraChrom** have been the alternative for the chromatography market.

On display are the 3 different types of STYROS® reversed phases offered compared with each other for the separation of 5 proteins of different size and different hydrophobicity.



Chromatogram 1
STYROS® 1R,2R,3R/NB Simulated-Monolith™
 Flow Rate: 0.2 ml/min.

Table 1. Operating parameters.

HPLC System.	Agilent 1290 Infinity with thermostatted column compartment.
Columns	STYROS® 1R,2R,3R/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, 350 cm/hr. in an empty column.
Gradient	25 to 50 % B in 15 minutes (2.5 column volume)
Temperature	37°C
Detection	214 nm
Injection volume	10 µl
Pressure Drop	Shown on chromatogram at the start of the gradient and including the system back pressure.
Sample:	Ribonuclease A, Insulin, Cytochrome c, Lysozyme, BSA. 1 mg/ml each in buffer A.

Despite the high performances, the back pressures of the columns are neither high nor prohibitive.

They are at the lower end of any HPLC or UHPLC system and can also be considered with FPLC systems.

The high organic solvents needed is also to be noted as it is one of the requirements for the hyphenation with Mass Spectrometers.

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

The column can be operated with up to 5,000 psi of pressure.

Leaching or the absence of it is an advantage to be considered when moving to production as it would represent the final step that no longer requires “polishing” often needed to remove the impurities that are the direct result of such deficiencies.

