

APPLICATION NOTE

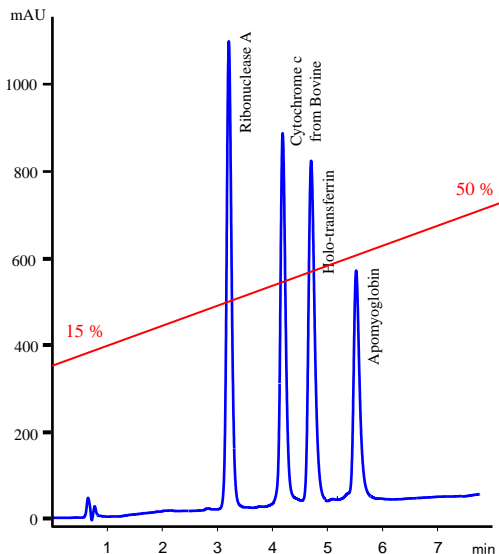
STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Comparing Narrow Bore column with a standard Bore of 4.6 mm ID.

Most major labs have now taken a responsible stand towards the environment.

The high cost of solvents as well as their disposal has been taken into consideration by opting towards smaller bore columns to minimize such impacts.

Furthermore, by moving in that direction, the end users have seen additional benefits such as higher sensitivity.

In the present application we are using a narrow bore column of 2.1 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace larger bore columns of 4.6 mm ID.



Chromatogram 1

Separation of Standard proteins on **STYROS® 2R/NB**
Flow Rate: 0.2 ml/min

Table 1. Operating parameters.

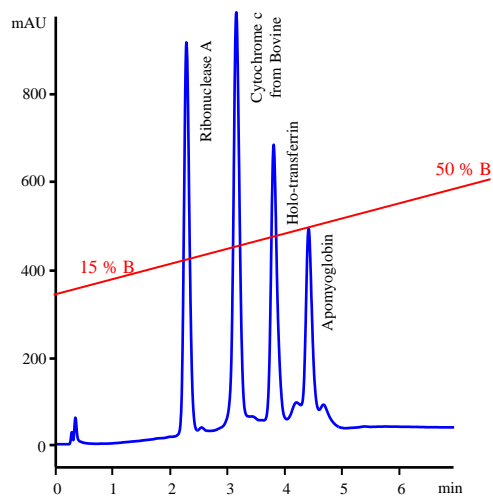
HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	STYROS® 2R/NB 2.1 X 50 mm
Mobile phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.2 ml/min
Gradient	15 to 50 % B in 8 min. (9 cv)
Temperature	40°C
Detection	220 nm
Injection volume	2 µl
Pressure Drop	27 bar (400 psi)
Sample:	Protein Standard from Sigma: Ribonuclease A, Cytochrome c from bovine, Holo-transferrin, Apomyoglobin, (1 mg/ml each in buffer A)

The media does not leach and can be used with mass spectrometer. The size of the column allows minimal splitting to the waste for the hyphenation.

Such uses are becoming more common as mass spectrometers are adopted as a way of both detecting and identifying a substrate with high precision.

The normal bore of 4.6 mm ID with the same length requires 25 µl of the same sample to produce the same level of detection compared with 2 µl in the case of the narrow bore column.

The amount of solvent used for the 7 minutes run in this case is 17.5 ml as compared with the 1.6 ml with the narrow bore column.



Chromatogram 2

Separation of Standard proteins on **STYROS® 3R/XH**
Flow Rate: 2.5 ml/min

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS® 3R/XH 4.6 X 50 mm
Mobile phase.	A: 0.1% TFA in H2O B: 0.1% TFA in ACN: H2O 95:5
Flow rate	2.5 ml/min
Gradient	15 to 50 % B in 5 min. (15 cv)
Temperature	30°C
Detection	220 nm
Injection volume	25 µl
Pressure Drop	12 bar (174 psi)
Sample:	Protein Standard from Sigma: Ribonuclease A, Cytochrome c from bovine, Holo-transferrin, Apomyoglobin, (1 mg/ml each in buffer A)

