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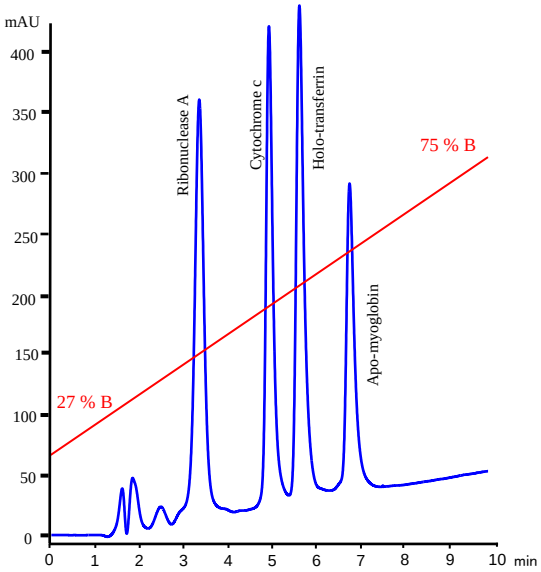
APPLICATION NOTE

STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Separation of 5 proteins standard on Micro Bore column of 1 mm ID. Comparison with Narrow Bore of 2.1 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 Micro Bore column of 1 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace Narrow Bore columns of 2.1 mm ID.



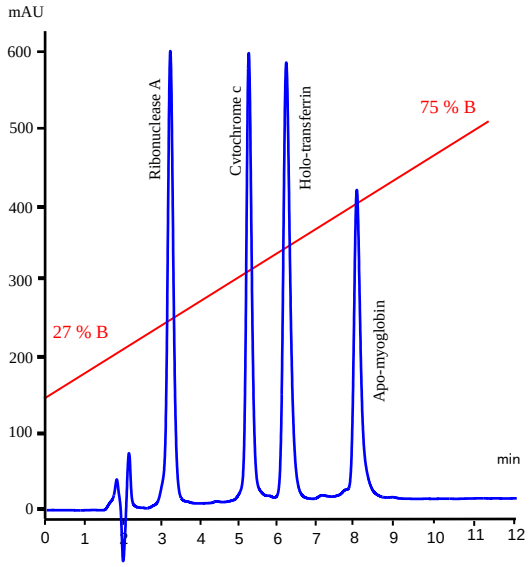
Chromatogram 1
Separation of 4 Standard proteins on **STYROS® 2R**/MB
Flow Rate: 0.1 ml/min.

Table 1. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R**/MB 1 X 300 mm
Mobile phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.1 ml/min.
Gradient	27 to 75 % B in 10 minutes (~ 6 cv)
Temperature	60°C
Detection	214 nm
Injection volume	1 µl
Pressure Drop	124 bar (~1800 psi) at the start of gradient
Sample:	Protein Standard from Sigma: as indicated on the chromatogram

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.

Compared with the Narrow Bore column, 60 % less of eluent is used and 50 % less of sample needed.



Chromatogram 2
Separation of 4 Standard proteins on **STYROS® 2R**/NB
Flow Rate: 0.2 ml/min.

Table 2. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R**/NB 2.1 X 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.
Gradient	27 to 75 % B in 12 minutes (~7 cv)
Temperature	60°C
Detection	214 nm
Injection volume	2 µl
Pressure Drop	49 bar (~700 psi)
Sample:	Protein standard from Sigma: as indicated on the chromatogram

As Simulated-Monolith™ the separations can be run at high linear velocities as noted above.
It is important to keep in mind the dwell volume of the instrument when using small bore columns as too large of a dwell volume is not helpful in properly achieving the required gradient.