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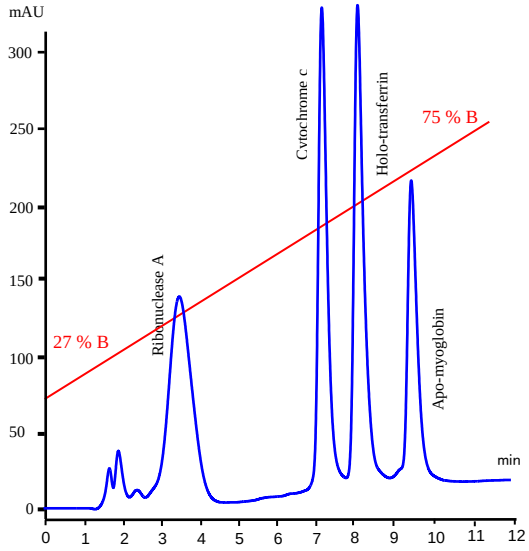
APPLICATION NOTE

STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Separation of 4 proteins standard on Capillary column of 0.5 mm ID. Comparison with Micro Bore of 1 mm ID.

The improvement of mass spectrometers has reached a point where the injection of a mixture allows the detection of its components without the need of any prior separation on an LC column.
The focus is now the contamination of the samples as a result of leaching of the LC columns.

In the present application we are using a Capillary column of 0.5 mm ID and suggest STYROS® polymeric media as Simulated-Monolith™ to replace Micro Bore columns of 1 mm ID.

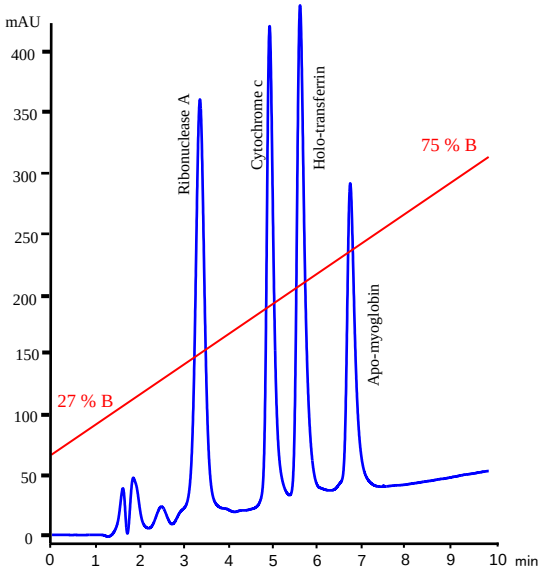


Chromatogram 1
Separation of 4 Standard proteins on **STYROS® 2R**/Cap
Flow Rate: 0.04 ml/min.

Table 1. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R/Cap** 0.5 X 300 mm
Mobile phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.04 ml/min.
Gradient	27 to 75 % B in 12 minutes (~ 7 cv)
Temperature	60°C
Detection	214 nm
Injection volume	0.5 µl
Pressure Drop	160 bar (~ 2300psi) 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 Micro Bore column, 50 % less of eluent and sample are needed for the separation.



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

Table 2. 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)
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 so does the column regeneration.
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.

Inc.