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The Vanguard of Liquid Chromatography.

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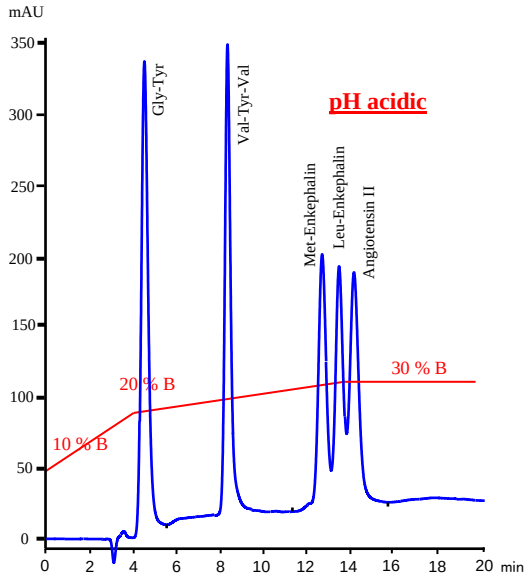
APPLICATION NOTE

STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Separation of 5 peptides on Micro Bore column of 1 mm ID. Acidic and Basic pH's.

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.



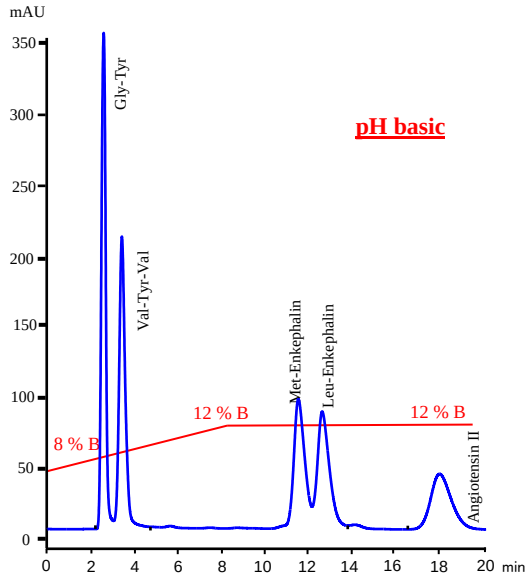
Chromatogram 1
Separation of 5 Standard peptides on **STYROS® 2R**/MB
Flow Rate: 0.06 ml/min.

Table 1. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R**/MB 1 X 150 mm
Mobile phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN: H2O 95:5
Flow rate	0.06 ml/min.
Gradient	10 to 20 % B in 4 min (1.1 cv), to 30% B in 14 minutes (2.8 cv)
Temperature	60°C
Detection	220 nm
Injection volume	0.5 µl
Pressure Drop	70 bar (1015 psi) at the start of gradient
Sample:	Peptide 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.

A total of 1.2 ml of eluents were consumed or waste generated for the separation and 0.5 µl of sample.



Chromatogram 2
Separation of 5 Standard peptides on **STYROS® 2R**/NB
Flow Rate: 0.06 ml/min.

Table 2. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS ®2R**/MB 1 X 250 mm
Mobile phase.	A: H2O with 20 mM NH4OH, pH=12 B: A + 95 % ACN
Flow rate	0.06 ml/min.
Gradient	8 to 12 % B in 8 min (2.3 cv), 12 % B to 10 minutes
Temperature	60°C
Detection	220 nm
Injection volume	0.5 µl
Pressure Drop	42 bar (609 psi)
Sample:	Peptide Standard from Sigma: as indicated on the chromatogram

Depending on the product of interest, the separation can be run in acidic or basic pH without any concern for the stability or instability of the media.
No concern either for the temperature or pore size as the concept of pore size does not apply to monoliths or Simulated-Monoliths™.
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.