OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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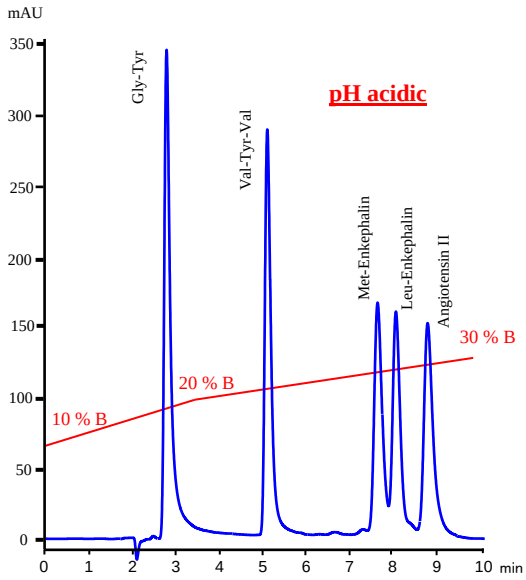
APPLICATION NOTE

STYROS® 2R Simulated-Monolith™ Polymeric Reversed Phase. Separation of 5 peptides on Narrow Bore column of 2.1 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 narrow bore column of 2.1 mm ID for the separation of 5 standard peptides and suggest STYROS® polymeric media as Simulated-Monolith™ to replace larger bore columns of 4.6 mm ID.

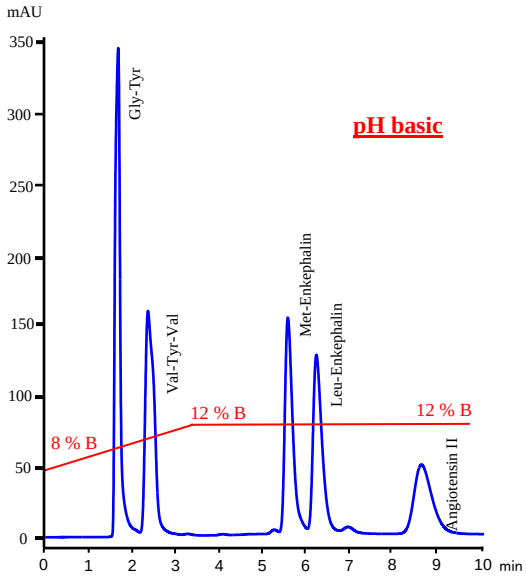


Chromatogram 1
Separation of 5 Standard peptides on **STYROS® 2R**/NB
Flow Rate: 0.2 ml/min.

Table 1. Operating parameters.

HPLC System.	Agilent 1290 with thermostatted column compartment.
Columns	**STYROS® 2R**/NB 2.1 X 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	10 to 20 % B in 3 min (1.2 cv), to 30% B in 10 minutes (2.7 cv)
Temperature	60°C
Detection	220 nm
Injection volume	1 µl
Pressure Drop	42 bar (609 psi)
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 2 ml of eluents were consumed during the separation along with 1 µl of sample.



Chromatogram 2
Separation of 5 Standard peptides 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: H2O with 20 mM NH4OH, pH=12 B: A + 95 % ACN
Flow rate	0.2 ml/min
Gradient	8 to 12 % B in 3 min (1.2 cv), 12 % B to 10 minutes (2.7 cv)
Temperature	60°C
Detection	220 nm
Injection volume	1 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™.

Inc.