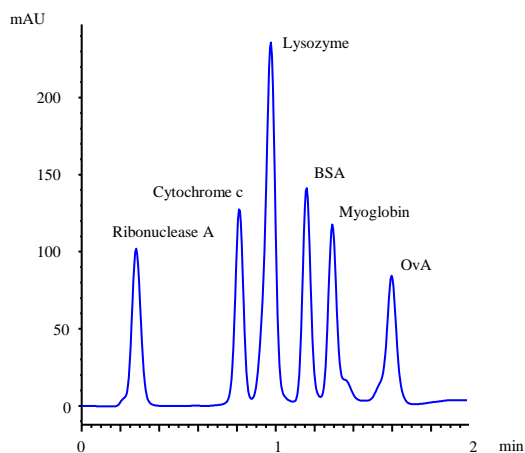


APPLICATION NOTE

STYROS™ 3R Simulated Monolith™ Polymeric Reversed Phase.

Hard gel polymeric gigaporous stationary phases offer similar mechanical stability compared to silica and far superior chemical stability regarding extremes of pH's.

The present application note highlights the capabilities of **STYROS™ 3R Simulated Monolith™** in the separation of biomolecules as well as smaller peptides and other molecules under different and extreme conditions.

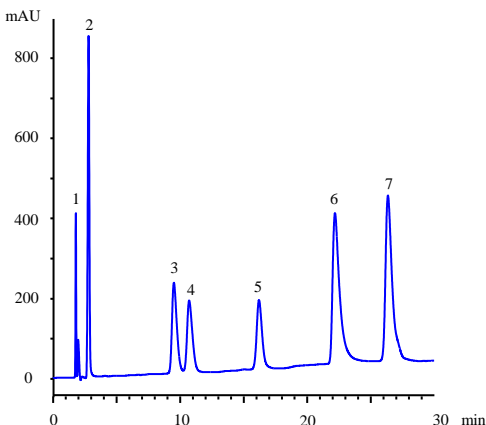


Chromatogram 1

Separation of 6 proteins on **STYROS™ 3R/XH**
(Flow Rate: 4 ml/min)

Table 1. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ 3R/XH 4.6 X 50 mm
Mobile phase.	A: 0.075% TFA in H ₂ O B: 0.075% TFA in ACN:H ₂ O 95:5
Flow rate	4 ml/min (1,450 cm/hr of linear flow rate)
Gradient	25 to 62 % B in 2 min. (9.6 cv)
Temperature	30°C
Detection	220 nm
Injection volume	2.5 µl
Sample:	Ribonuclease A, Cytochrome c, Lysozyme, BSA, Myoglobin, OVA (1 mg/ml each in buffer A)

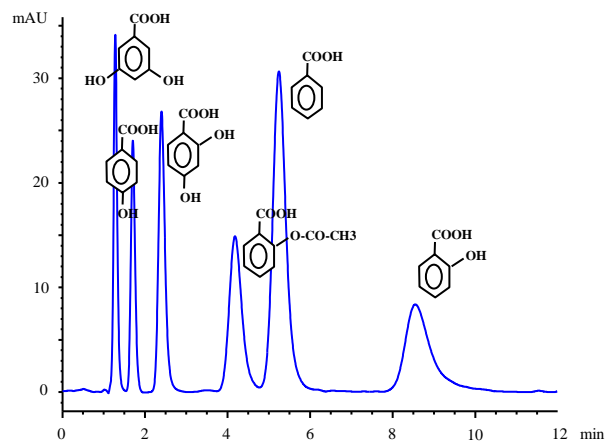


Chromatogram 2

Separation of 7 peptides on **STYROS™ 3R/XH**
(Flow Rate: 1 ml/min)

Table 2. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ 3R/XH 4.6 X 250 mm
Mobile phase.	A: 10 mM Phosphate in H ₂ O, pH= 11.2 B: ACN
Flow rate	1 ml/min (360 cm/hr of linear flow rate)
Gradient	5 to 15 % B in 30 min. (7.2 cv)
Temperature	30°C
Detection	220 nm
Injection volume	100 µl
Sample:	1. Gly-Tyr, 2. Val-Tyr-Val, 3. Met enkephalin, 4. Leu enkephalin, 5. Angiotensin II, 6. Angiotensin III, 7. Angiotensin I (0.5 mg/ml each in DI H ₂ O)



Chromatogram 3

Separation of Benzoic acid and its derivatives on **STYROS™ 3R/XH**
(Flow Rate: 1.5 ml/min)

Table 3. Operating parameters.

HPLC System.	Agilent 1100 with thermostatted column compartment.
Columns	STYROS™ 3R/XH 4.6 X 100 mm
Mobile phase.	A: 10 mM Phosphate, pH=1.5 B: ACN
Flow rate	1.5 ml/min (540 cm/hr of linear flow rate)
Gradient	Isocratic: 15 % B
Temperature	30°C
Detection	230 nm
Injection volume	0.5 µl
Sample:	1. 3,5-Dihydroxybenzoic acid, 2. 4-Hydroxybenzoic acid, 3. 2,4-Dihydroxybenzoic acid, 4. Acetyl Salicylic acid, 5. Benzoic acid, 6. Salicylic acid, (170 ug/ml each in A:B 50:50)

Unlike Monolith **STYROS™ Simulated Monolith** columns are available in many sizes for additional resolving capabilities.

