

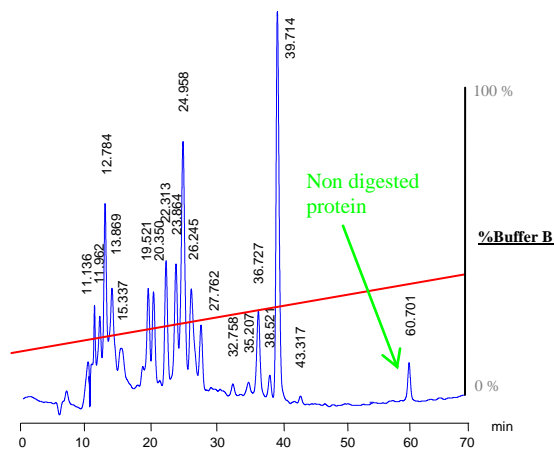
APPLICATION NOTE

StyrosZyme™ Papain, Immobilized Enzyme on Simulated Monolith Polymeric Stationary Phase: Effect of Temperature and Linear Velocity on Online Digestion.

The chemical and mechanical stability of **STYROS™** Simulated Monolith provides the optimum support for the full range of immobilized components such as enzyme.

The present application note shows the practical aspect of the resulting products commercialized by **OraChrom** under the name **StyrosZyme™**.

During optimum conditions a **StyrosZyme™ Papain** column can digest over 99 % of a Cytochrome c sample during an online digestion in 4 minutes. The following chromatogram shows the resulting peptides mapped on a **STYROS™ 2R/XH** column placed in tandem after the enzyme column.



Chromatogram 1

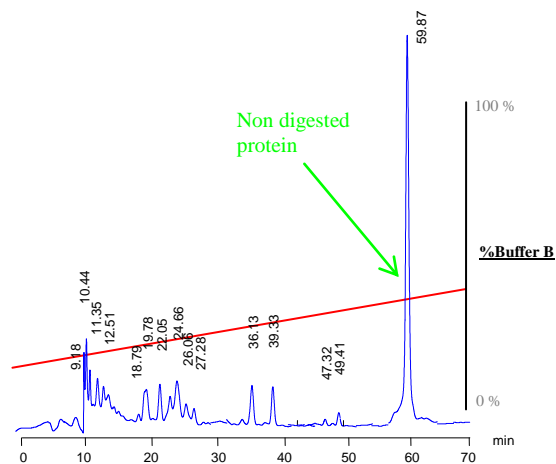
Peptide digest from Cytochrome c separated on a **STYROS™ 2R/XH** 4.6 X 250 mm. Digestion flow rate 0.15 ml/min. Temperature 60 °C.

Operating parameters for the chromatograms.

HPLC System.	Agilent 1100
Columns	StyrosZyme™ Papain 4.6 x 33 mm STYROS™ 2R/XH 4.6 X 250 mm
Mobile Phase For reversed phase.	A: 0.1% TFA in H ₂ O B: 0.1% TFA in ACN:H ₂ O (95:5)
Digestion buffer.	20 mM Phosphate, 20 mM Cysteine-HCl, 10 mM EDTA, at pH=6.2.
Detection	214 nm
Gradient for Separation	12 to 37 % B in 70 min @ 0.5 ml/min flow rate
Injection volume	8 µl
Sample:	10 mg/ml Cytochrome c from horse heart in 0.1 % TFA.

By modifying the temperature of the **StyrosZyme™ Papain** column, or changing the flow rate during the on line digestion, one can alter and control the extent of digestion.

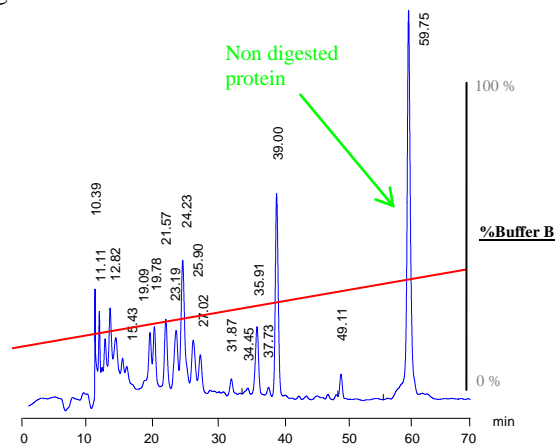
In the first example the temperature was decreased from 60°C to 37°C. As a result the digestion was reduced from 99% to 70% of the total protein injected on the enzyme column.



Chromatogram 2

Peptide digest from Cytochrome c separated on a **STYROS™ 2R/XH** 4.6 X 250 mm. Digestion flow rate 0.15 ml/min. Temperature 37 °C.

By increasing the flow rate from 0.15ml/min to 0.2ml/min during digestion, only 80% of the injected protein is digested.



Chromatogram 3

Peptide digest from Cytochrome c separated on a **STYROS™ 2R/XH** 4.6 X 250 mm. Digestion flow rate 0.2 ml/min. Temperature 60 °C.