OraChrom, Inc.
The Vanguard of Liquid Chromatography.

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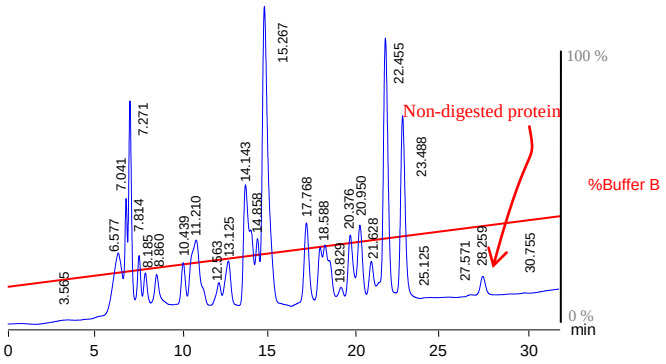
APPLICATION NOTE

StyrosZyme™ Pepsin, Immobilized Enzyme on Polymeric Hard Gel Stationary Phase: On line digestion of Cytochrome c From Horse Heart and Bovine Heart. Alternative.

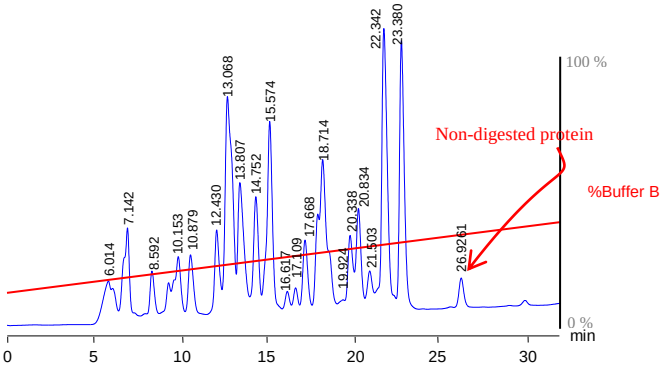
The chemical and mechanical stability of polymeric hard gel media that are the features of StyrosZyme™ immobilized enzyme columns, allow their use on line in a flow through setting.
The advantages resulting from such setting are numerous:

- Digestion time is reduced to a few minutes as compared to hours.
- The enzyme cartridge can be used as a direct inlet to either a LC or a MS system for the analysis of the resulting peptides, substantially reducing and simplifying the sample handling process and allowing it to be fully automated.
- The extent of digestion can be controlled by changing the flow rate and the temperature as well as the column length. It can also be made fully reproducible.
- The immobilized enzyme displays high stability towards low pH's, high flow rates, temperatures and back pressures.
- The possibility of using fast flow rates allows the cartridge to be reconditioned quickly, further reducing the process time.
- No auto-digestion occurs due to the absence of contact between enzyme molecules in the immobilized format.
- A single cartridge can be used during many digestions without losing its activity.
- The ratio of Enzyme to Substrate can be controlled in order to obtain target peptide in different concentrations.
- The pH can be controlled and the effect studied so does the temperature.

In the following examples Cytochrome c from two different sources were used to highlight the variations in peptide profiles obtained during reversed phase mapping of the resulting digests.
Similar conditions are used in both cases to achieve over 99% digestion.
A typical digestion consists of running a known amount of protein in the StyrosZyme™ Pepsin column at flow rates of 50 µl/min (18 cm/hr for a 4.6 mm ID column). The resulting peptide digests are dumped into a reversed phase column connected in series with the StyrosZyme™ Pepsin column.
The enzyme column is then taken off line and the peptide mapped following a gradient.



Chromatogram 1
Peptide digests from Cytochrome c from bovine heart separated on a STYROS™ 2R/XH 4.6 X 150 mm at 0.5 ml/min (180 cm/hr)



Chromatogram 2
Peptide digests from Cytochrome c from horse heart separated on a STYROS™ 2R/XH 4.6 X 150 mm at 0.5 ml/min (180 cm/hr)

Table 1. Operating parameters for the chromatograms.

HPLC System.	Agilent 1100
Columns	StyrosZyme™ Pepsin 2.1 x 50 mm STYROS™ 2R/XH 4.6 X 150 mm
Mobile Phase For reversed phase.	A: 0.075% TFA in H2O B: 0.075% TFA in ACN:H2O (95:5)
Mobile Phase For Digestion.	100 mM Phosphate + 150 mM NaCl in DI H2O at pH=2.5.
Flow rate	As indicated.
Gradient	As indicated
Temperature	30°C
Detection	214 nm
Injection volume	5 µl
Sample:	10 mg/ml Cytochrome c in mobile phase buffer A.