OraChrom, Inc.
The Vanguard of Liquid Chromatography.

10-B Henshaw Street Woburn, MA 01801 USA
Phone (781) 932 0151 Fax (781) 932 0787
E-mail: ***info@orachrom.com*** ***www.orachrom.com***

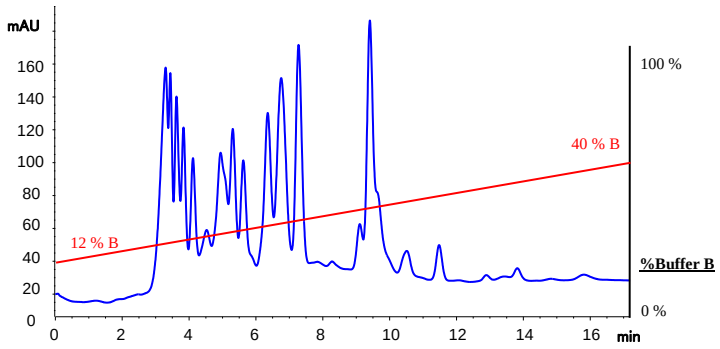
APPLICATION NOTE

StyrosZyme® Pepsin, Immobilized Enzyme on Simulated-Monolith™ Polymeric Hard Gel: Full on line digestion of a solution of 10 µl of 10 mg/ml protein.

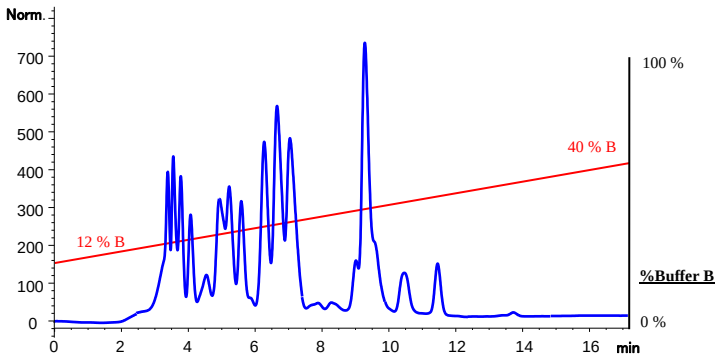
The chemical and mechanical stability of polymeric hard gel media that are the features of Simulated-Monolith™ StyrosZyme® immobilized enzyme columns, allow their use on line in a flow through setting.
The advantages resulting from such setting are numerous:

- Digestion time is reduced to a few minutes as compared to hours.
- The enzyme cartridge can be used as a direct inlet to either an LC or an 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. 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 of the enzymes occurs due to the absence of their contact 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.

In the following examples, different amounts of Cytochrome c were digested on a Narrow Bore column of 2.1 mm ID and 5 cm length.
A typical digestion consists of running a known amount of protein in the StyrosZyme® Pepsin column at flow rates of 100 and 25 µl/min. 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.
The process is fully automated using a series of sequence with the Open Lab control of Agilent. To digestion runs are fully automated.
It is important to note that the high retentive properties of the STYROS® polymeric media allows the capture of the resulting peptides. Higher amounts of organic are also needed to elute the captured peptide.



Chromatogram 1
Peptide digests from 2 µl Cytochrome c from horse heart separated on a **STYROS**® 3R/XH 4.6 X 150 mm at 1 ml/min



Chromatogram 2
Peptide digests from 10µl Cytochrome c from horse heart separated on a **STYROS**® 3R/XH 4.6 X 150 mm at 1 ml/min

Table 1. Operating parameters for the chromatograms.

HPLC System.	Agilent 1290 Infinity
Columns	**StyrosZyme**® Pepsin 2.1 x 50 mm **STYROS**® 3R/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 PO4H2Na + 150 mM NaCl in DI H2O at pH=2.5.
Flow rate	As indicated.
Gradient	As indicated
Temperature	37°C
Detection	214 nm
Injection volume	2 and 10 µl
Sample:	10 mg/ml Cytochrome c in mobile phase buffer A.