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* *orachrom.com*

APPLICATION NOTE

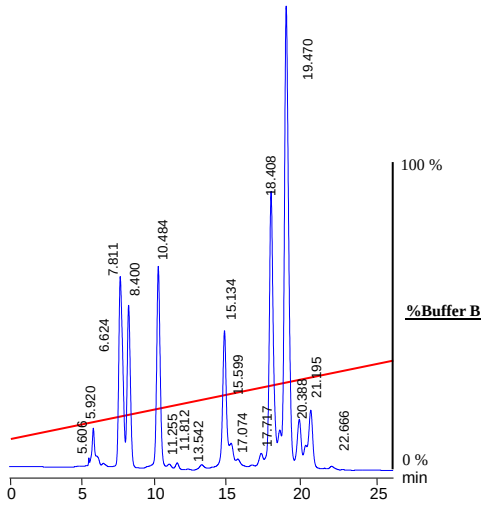
StyrosZyme™ TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Stationary Phase: Online Digestion of Lysozyme in 5 minutes.

The manufacturers of immobilized enzyme had always in mind automation of processes as the ultimate goal.
Soft gel media such as agarose have been used to effectively reduce the autodigestion. However, they are not an appropriate stationary phase for packing small bore columns that can withstand high backpressures. Silica as another alternative is not stable at high pHs required for protein digestions.
Hard gel, fully pervious polymeric such as **STYROS**™ media, combine soft gel's high surface and silica's high rigidity.
STYROS™ is made of highly crosslinked polystyrene-divinyl benzene gigaporous matrix that can withstand backpressures of up to 10,000 psi, as well as extremes of pHs. The chemical and mechanical stability 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 product commercialized by **OraChrom** under the name **StyrosZyme™ TPCK-Trypsin**.
The present set up consists of the **StyrosZyme**™ column (2.1 x 50 mm) connected in tandem with **STYROS**™ 2R/XH (4.6 x 250 mm), also reversed phase polymeric stationary phase that can withstand the same high pH's used during protein digestion.
A known amount of Lysozyme is injected into the **StyrosZyme**™ column at 100 µl/min of flow rate for 2 minutes. The flow rate is stopped for another 2 minutes. The digest is then flushed into the reversed phase column at 1 ml/min for 1 minute. After switching the enzyme column off line, the reversed phase column is equilibrated with 97 % buffer A, 3 % buffer B at 3 ml/min flow rate until stable baseline. The flow rate is then reduced to 1 ml/min and the resulting peptides are mapped following the indicated gradient.
The enzyme digest can alternatively be injected into a mass spectroscopy chamber either directly or after a preliminary separation on the reversed phase column. The possibility of using mass friendly buffer components with polymeric stationary phases does facilitate the LC-MS hyphenation.

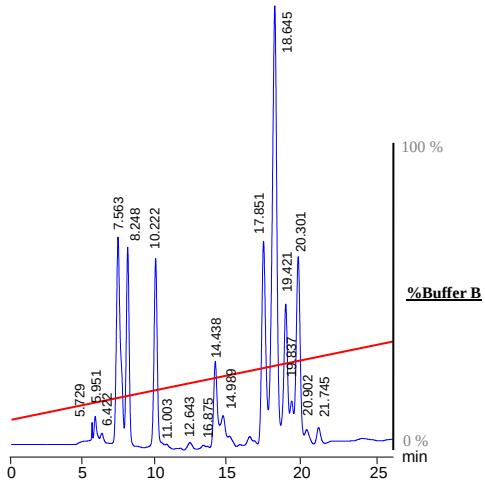
Table 1. Operating parameters for the chromatograms.

HPLC System.	HP 1100
Columns	**StyrosZyme**™ TPCK-Trypsin 2.1 x 50 mm **STYROS**™ 2R/XH 4.6 X 250 mm
Mobile Phase For reversed phase.	A: 0.1% NH4OH in H2O B: 0.1% NH4OH in ACN:H2O (95:5)
Mobile Phase For Digestion.	3 % ACN in 50 mM CO3HNH4 and CO3(NH4)2 aqueous buffer at pH=7.8 and 10 respectively..
Flow rate	As indicated.
Gradient	7 to 34 % B in 27 min.
Temperature	37°C
Detection	214 nm
Injection volume	10 µl
Sample:	10 mg/ml Lysozyme in 0.1 % TFA.

The following two chromatograms depict the effect of pH on the digestion.



Chromatogram 1
Peptide map from Lysozyme digest at ***pH = 7.8*** separated on a **STYROS**™ 2R/XH (4.6 X 250 mm reversed phase) at 1 ml/min.



Chromatogram 2
Peptide map from Lysozyme digest at ***pH = 10*** separated on a **STYROS**™ 2R/XH (4.6 X 250 mm reversed phase) at 1 ml/min.