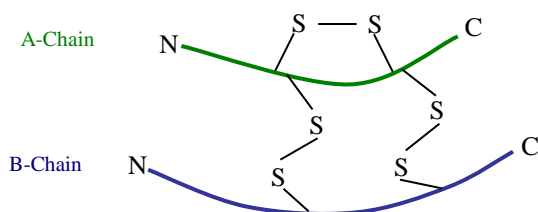


APPLICATION NOTE

StyrosZyme™ TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Stationary Phase: Online Digestion of Insulin Oxidized B-chain in 16 minutes.

The active form of insulin consists of 2 chains connected by disulfide bonds between cysteine side chains:



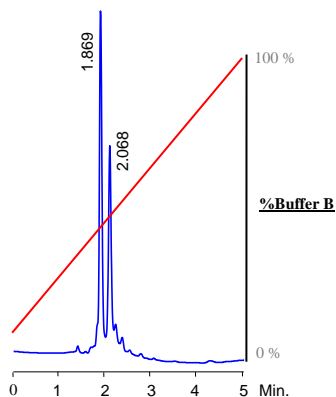
Oxidized B-Chain consists of the following amino-acid sequence:

Phe-Val-Asn-Gln-His-Leu-Cys-(SO₃H)-Gly-Ser-His-Leu-Val-Glu-Ala-Leu-Tyr-Leu-Val-Cys-(SO₃H)-Gly-Glu-Arg-Gly-Phe-Phe-Tyr-Thr-Pro-Lys-Ala

A typical digestion in solution with high grade Trypsin requires a minimum of **18 hrs** at 37 °C.

StyrosZyme™ TPCK-Trypsin can achieve the same result on line, in **16 minutes** at 37 °C.

The resulting peptides can be mapped directly after digestion by using STYROS™ 2R reversed phase column connected in series to the immobilized enzyme column in less than 3 minutes.



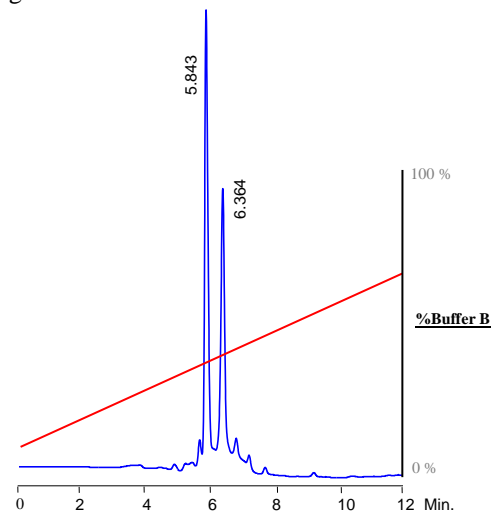
Chromatogram 1

Peptide digests from Insulin oxidized B-chain separated on a **STYROS™ 2R/XH 4.6 X 150 mm** at 3 ml/min (1,100 cm/hr)

The set up consists of a StyrosZyme™ TPCK-Trypsin column (2.1 x 100 mm), in tandem with the reversed phase polymeric STYROS™ 2R/XH (4.6 X 150 mm) column, capable to withstand long term high pH's.

A known amount of Insulin Oxidized B-chain in solution is injected into the immobilized enzyme column and run for 14 minutes at 25 µl/minute, followed by 2 minutes at 1 ml/min. The effluents from the StyrosZyme™ TPCK-Trypsin column are directly deposited onto the polymeric STYROS™ 2R/XH column. The enzyme column is then switched off line. The reversed phase column is equilibrated and the resulting peptides are mapped by developing a gradient as indicated on the chromatograms. The entire digestion and mapping can be automated and run in less than 30 minutes including the re-equilibration of both columns.

Slower flow rates minimally affect the resolution as shown in chromatogram 2.



Chromatogram 2

Peptide digests from Insulin oxidized B-chain separated on a **STYROS™ 2R/XH 4.6 X 150 mm** at 1 ml/min (360 cm/hr)

Table 1. Operating parameters for the chromatograms.

HPLC System.	HP 1100
Columns	StyrosZyme™ TPCK-Trypsin 2.1 x 100 mm STYROS™ 2R/XH 4.6 X 150 mm
Mobile Phase For reversed phase.	A: 0.1% NH ₄ OH in H ₂ O B: 0.1% NH ₄ OH in ACN:H ₂ O (95:5)
Mobile Phase For Digestion.	3 % ACN in 100 mM Tris-HCl aqueous buffer at pH=8.5.
Flow rate	As indicated.
Gradient	As indicated
Temperature	37°C
Detection	214 nm
Injection volume	10 µl
Sample:	3 mg/ml Insulin oxidized B-chain in buffer A.