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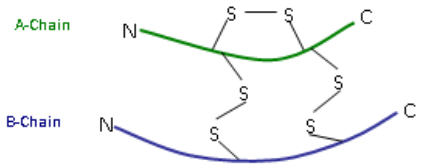
APPLICATION NOTE

Automated Digestion with StyrosZyme® TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Simulated-Monolith™ Enzyme Reactor.
Use of a 10 port valve to minimize salt exposure to the Mass. Spectrometer.

In this Application Note we have explored the use of Narrow Bore columns as well as a 10-port switching valve for the 1290 Infinity from Agilent to reduce the volume of solvent as well as the amount of substrate to be digested while at the same time allowing the process being automated and avoiding the contamination of the mass spectrometer with salt during the digestion process.

Oxidized Insulin B Chain is one of the two chains forming the active form of insulin:



It 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 of insulin in solution with high grade Trypsin requires at least 18 hours at 37 °C.
StyrosZyme® TPCK-Trypsin can achieve the digestion on line at 37°C, in 2 minutes at 0.5 ml/min. That is a linear velocity of close to 900 cm/hr. based on an empty column.

The digestion and mapping proceeds in under 30 minutes.

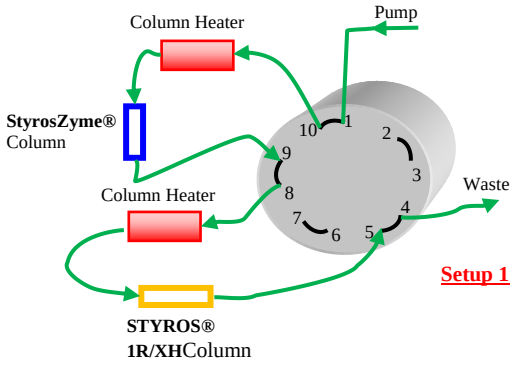
The resulting peptides can be mapped directly after digestion by using a STYROS®1R reversed phase column connected in series to the immobilized enzyme column in10 minutes.

The 3 buffers needed are as follows:
Buffer A: 0.1 % Formic acid in DI H2O (for peptide mapping)
Buffer B: 0.1 % Formic acid in Acetonitrile: H2O, 95:5(for peptide mapping)
Buffer C: 0.1 M TRIS, 0.1 M NaCl, pH= 8.5 (for digestion)

The temperature is set at 37°C for all sequences

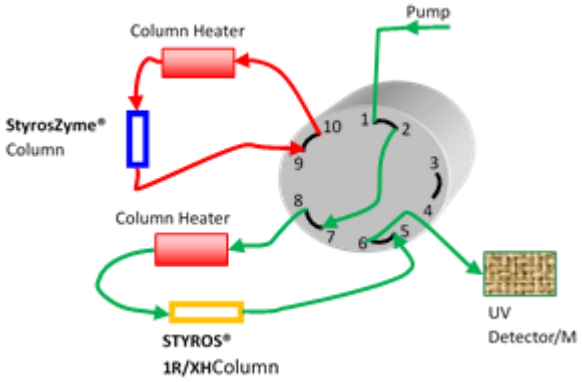
The setups for the automated digestion are described below.

A 10-port valve connects the enzyme column to the polymeric reversed phase STYROS® 1R column in series as shown in the schematic diagrams of Setup 1. The effluents at this stage are directed to waste.



Setup 1

In the second position, the enzyme column is removed from the line and only the reversed phase STYROS® 1R column remains.



Setup 2

A Narrow Bore enzyme column of 2.1x100 mm stainless Steel (StyrosZyme® TPCK-Trypsin) is used.
The reversed phase column (STYROS® 1R) is also a Narrow Bore column of the same size.

As a polymeric reversed phase Simulated-Monolith™ column, it can withstand extremes of pHs and has low pressure drops.
A sequence consisting of 6 steps is used in the following order:

1-Equilibrate the enzyme column with both columns in line as shown in Setup 1.

Time	% of buffer C	Flow rate (ml/min)
0		0
0.01	100	1
2	100	1

2-With both columns in line as in Setup 1, 5, 2 and 0.5 µl of a solution of 3 mg/ml of Insulin chain B in buffer A are injected and the resulting digests are dumped on the reversed phase column using the following method:

Time	% of buffer C	Flow rate (ml/min)
0		0
0.01	100	0.5
2	100	0.5

3-The reversed phase column only is now in line as in Setup 2 to wash the leftover salt and prepare it for the reversed phase mapping.
It is also ready for hyphenation with a mass spectrometer.

Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0
0.01	5	95	1
6	5	95	1

4-The digested peptides are now trapped on the reversed phase column and can be mapped following a gradient.
The setup is now as Setup 2.

Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0
0.01	5	95	0.4
6	90	10	0.4
10	90	10	0.4

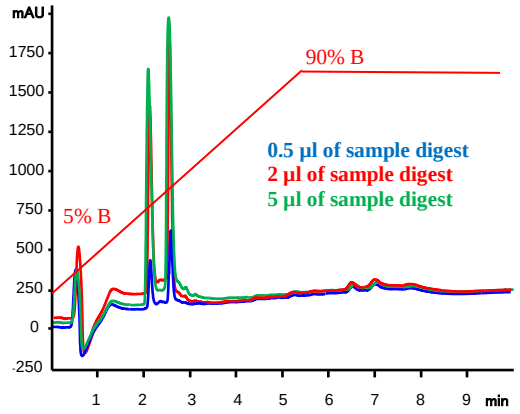
5- The reversed phase column is now preequilibrated to the initial low organic prior to getting in contact with the digestion buffer.
The setup remains as Setup 2.

Time	% of buffer B	% of buffer A	Flow rate ml/min
0			0
0.01	5	95	0.6
3	5	95	0.6

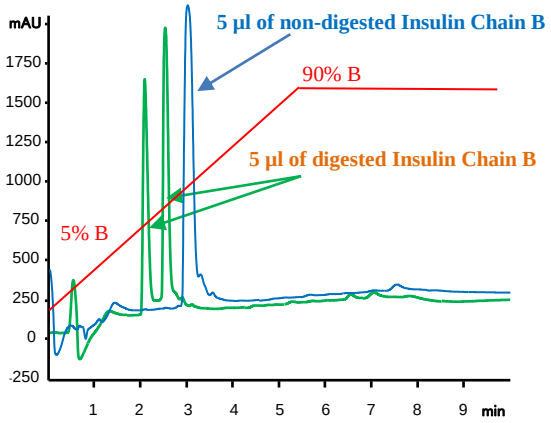
6-In the final step the line and the reversed phase column are flushed clean from any leftover organic going to the enzyme column. The setup therefore remains as setup 2.

Time	% of buffer C	Flow rate (ml/min)
0		0
0.01	100	0.6
4	100	0.6

The result of the automated digestion and mapping of the immobilized enzyme (TPCK-Trypsin) in a 2.1x100 mm column with different amounts of substrate is shown and compared to one another.



The digestion is complete from 0.5 to 5 µl of sample at 0.5 ml/min of flow rate with a Narrow Bore column.



The automation can now lend itself in getting results in real time of digestion needed to diagnose mutations in various diseases including cancer.

Inc.