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The Vanguard of Liquid Chromatography.

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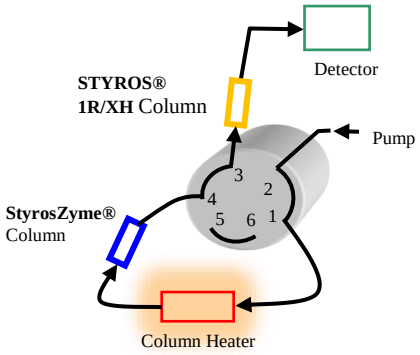
APPLICATION NOTE

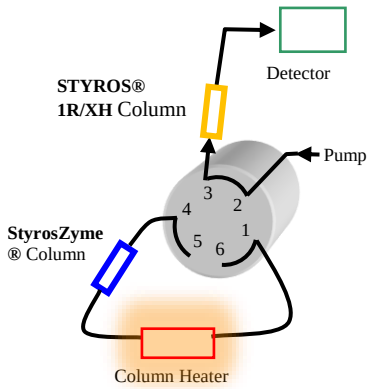
Automated Digestion with StyrosZyme® TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Simulated-Monolith™.
Digestion and mapping of 10 µg of Lysozyme on Narrow Bore column.

The Agilent 1290 Infinity was used for the automated digestion of Lysozyme and mapping of the resulting fragments.
Narrow Bore columns can extend the number of runs with the use of limited amount of solvent as well as minimum amount of sample.
The initial setting of the instrument as depicted below has both columns in series and on line:
During this stage the protein is digested and trapped on the polymeric reversed phase column.



Setup 1

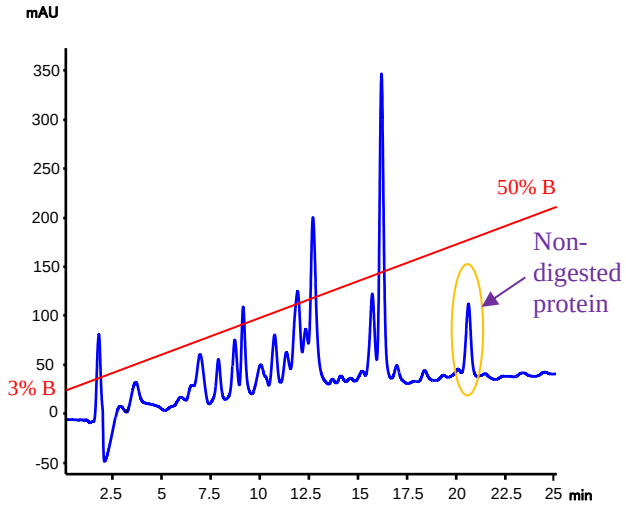
The second setting has only the reversed phase column for the mapping of the resulting peptides.



Setup 2

The digestion and mapping proceeds in under one hour.
Lysozyme from chicken egg white was used for this application.
To get over 95% digestion the flow rate on the StyrosZyme® TPCK-Trypsin column was reduced to 0.1 ml/min of volumetric flow that is a linear velocity of 174 cm/hr. based on an empty column.
Like the digestion of cytochrome c in the previous Application Note, Lysozyme is not treated, and it is in its native form. The digestion however is limited to lower flow rates.

The resulting peptides are trapped on a STRYROS® 1R polymeric reversed phase column to be desalted, mapped and ultimately hyphenated with a mass spectrometer.
The intent is not to get high-resolution separations, rather a sample that can be introduced into the mass chamber.



The enzyme column (StyrosZyme® TPCK-Trypsin) is a 2.1x100 mm Narrow Bore column followed by a 2.1x100 mm reversed phase (STYROS® 1R), also Narrow Bore reversed phase column to trap and map the resulting digested peptides.
As a polymeric reversed phase Simulated-Monolith™ column, it can withstand extremes of pH's and has low pressure drops.

The 3 buffers needed are as follows:
Buffer A: 0.075 % TFA in DI H2O (for peptide mapping)
Buffer B: 0.075 % TFA 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.
They consist of 6 steps 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	0.6
4	100	0.6
4.1	100	0.6

2-With both columns in line as in Setup 1, 1 µl of a solution of 10 mg/ml of Lysozyme in buffer A is injected and the resulting digests are dumped on the reversed phase column using the following sequence:

Time	% of buffer C	Flow rate (ml/min)
0	100	0
0.01	100	0.1
10	100	0.1

3- The enzyme column is removed as shown in Setup 2 to wash off the salt and prepare the reversed phase column for mapping. It will also be ready for the hyphenation with a mass spectrometer.

Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0.0
0.01	3	97	0.6
8	3	97	0.6

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

Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0	3	97	0.2
0.01	3	97	0.2
25	50	50	0.2

5- The reversed phase column is washed in this step to remove any leftover peptides from the previous digestion. The setup remains as Setup 2.

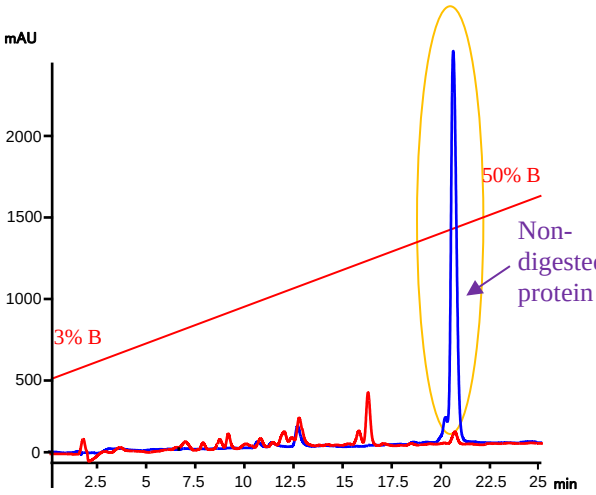
Time	% of buffer B	% of buffer A	Flow rate ml/min
0	10	90	0
0.01	55	45	0.5
0.5	90	10	0.5
1	90	10	0.5
1.5	45	55	0.5
1.6	3	97	0.5
6	3	97	0.5

6-In the final step the reversed phase column is pre-equilibrated with the digestion buffer to wash off all organics from the line.
The setup remains as Setup 2.

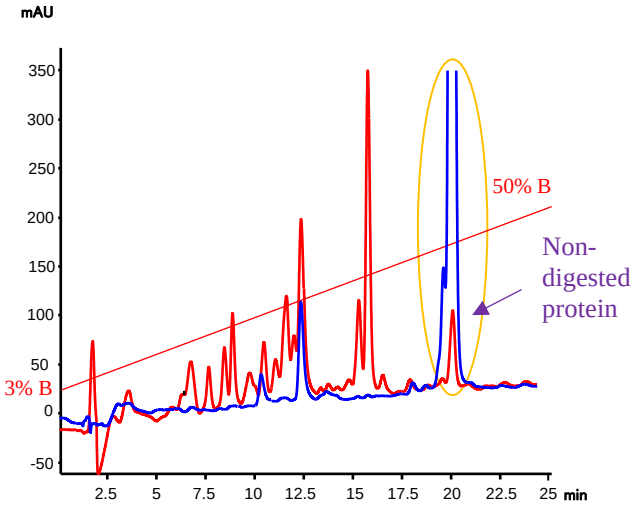
Time	% of buffer C	Flow rate (ml/min)
0		0.0
0.01	100	0.5
6	100	0.5

The pressure drop of the system does not exceed 300 bars even at 0.6 ml/min with both columns on line.
It is therefore safe to run multiple injections overnight with no concern of the system exceeding its pressure limit of 1300 bar at such flow rates.

To assess the extent of the digestion similar amount of Lysozyme (1 µl) was directly injected into the reversed phase column to assess all components that will contribute to the mapping of the resulting peptides.
In the present case the initial Lysozyme from chicken egg shows additional impurities.
The sample is assessed to be over 95% pure by SDS Page by the manufacturer.



Looking at the enlarged chromatogram of the same comparative runs:



The chromatogram shows the digestion to be over 95 % complete,

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