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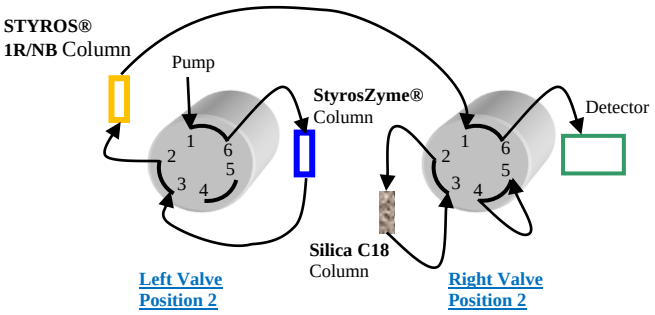
APPLICATION NOTE

Automated Digestion and Silica mapping with StyrosZyme® TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Simulated-Monolith™ Enzyme Reactor with the Acquity UPLC *I* class Plus.

Two 6 ports, two positions switching valve are used with the Acquity UPLC *I* class Plus in order to ultimately map the peptides resulting from the online digestion of Lysozyme on a Silica C18 column (Acquity UPLC® BEH C18 1.7 µm 2.1x50 cm column).
The 3 buffers needed are as follows:
Buffer A: 0.1 % DFA in DI H2O: ACN 98:2 (for peptide mapping)
Buffer B: 0.1 % DFA in ACN: H2O, 70:30 (for peptide mapping)
Buffer C: 0.1 M CO3HNH4, pH= 7.8 (for digestion)

A Narrow Bore enzyme column of 2.1x50 mm stainless Steel (StyrosZyme® TPCK-Trypsin) as well as a reversed phase Narrow Bore column (STYROS® 1R) column of the same size are used with Waters Acquity UPLC *I* class Plus with a 2 positions 6-port switching valve

The following shows the starting position of the valves used:



Setup 1

In this first position, the StyrosZyme® enzyme column and the polymeric STYROS® 1R/NB columns only, are in line and the Silica C18 column is not exposed to any high pH aqueous buffer that is used for the digestion.
It is therefore possible to safely scout the right digestion buffer as well as the optimum conditions for the full digestion prior to the use of the silica column.

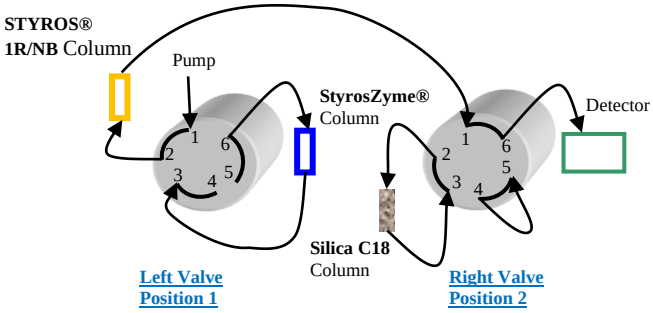
1-Equilibrate the enzyme column with both columns in line as shown in Setup 1.

Time	% of buffer C	Flow rate (ml/min)
0		0
0.01	100	0.2
6	100	0.2

2-With both columns in line as in Setup 1, the Lysozyme sample is 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	0.1
8	100	0.1

In the second set up, the left valve is switched to position 1 still with only the STYROS® 1R reversed phase column on line in order to equilibrate it with the starting solvent gradient.



Setup 2

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	0	100	0.3
10	0	100	0.3

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

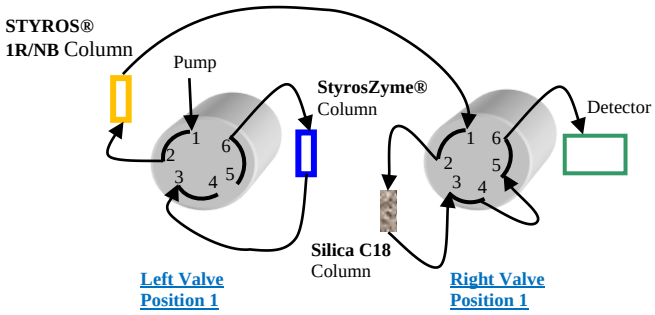
Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0
0.01	0	100	0.2
60	60	40	0.2

Once the conditions of the digestion are satisfactory, one can use the second switching valve to bring the Silica C18

column in line as shown in Setup 3.
The same gradient as in step 4 can be used for the mapping to run the separation on the C18 Silica column:

4'-The digested peptides trapped on the polymeric reversed phase column can now be mapped on the C18 Silica column. The setup is now as Setup 3.

Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0
0.01	0	100	0.2
60	60	40	0.2



Setup 3

To re-equilibrate both reversed phase columns that are now in line, a similar step than step 5 is used.

5- Both reversed phase columns are preequilibrated to the initial low organic prior to getting in contact with the digestion buffer.
The setup is now Setup 3.

Time	% of buffer B	% of buffer A	Flow rate ml/min
0			0
0.01	0	100	0.2
6	0	100	0.2

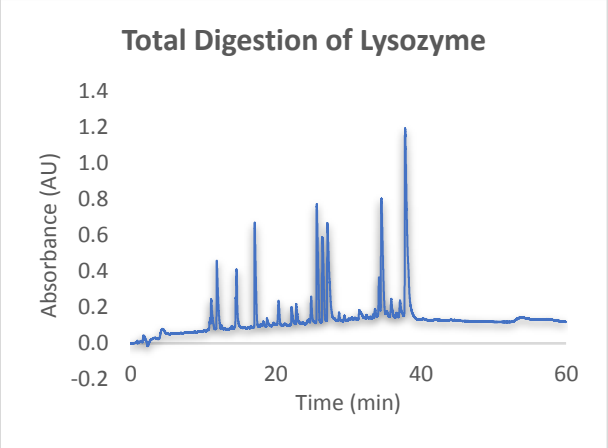
6-In the final step the line and the reversed phase column are flushed clean from any leftover organic going to the enzyme column, still avoiding the Silica column. The setup therefore remains as setup 2.

Time	% of digestion buffer C	Flow rate (ml/min)
0		0
0.01	100	0.2
6	100	0.2

The system is now ready for the next cycle to check the reproducibility of the digestion.

The use of Narrow Bore column requires minimal use of solvents therefore the automated digestion and mapping can be run around the clock without any concern of running out of buffers.
We have used 1µl of a solution of 10 mg/ml of Lysozyme from chicken egg as sample to digest.

The temperature is set at 37°C for all sequences.
The digestion is complete, and it is not contaminated with any enzyme auto digest nor there are added weight resulting from alkylation and alike to prevent the substrate from refolding.
The shear convective force appears to be sufficient for the full digestion of the protein.



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 a LC or a 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 as well as the column length. It can also be made fully reproducible.
- The immobilized enzyme displays high stability towards low and high 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 occurs due to the absence of contact between enzyme molecules 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 to obtain target peptide in different concentrations.
- The pH can be controlled, and the effect studied so does the temperature.

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