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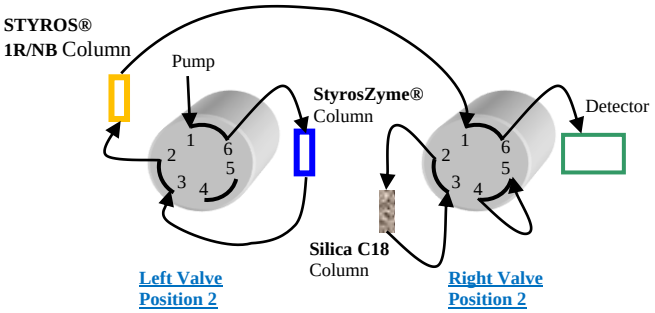
APPLICATION NOTE

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

Using the same setup as in the previous Application Note 141, Trypsin itself was used for the digestion.
The 3 buffers used are the same:
Buffer A: 0.1 % TFA in DI H2O: ACN 98: 2 (for peptide mapping)
Buffer B: 0.1 % TFA in ACN: H2O, 70:30 (for peptide mapping)
Buffer C: 0.1 M Tris, pH= 8.55 (for digestion).
The 3 columns include:
A Narrow Bore enzyme column of 2.1x50 mm stainless Steel (StyrosZyme® TPCK-Trypsin), with the same size reversed phase Narrow Bore column (STYROS® 1R) ending with the Silica C18 column (Acquity UPLC® BEH C18 1.7 µm 2.1x50 cm column).

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.

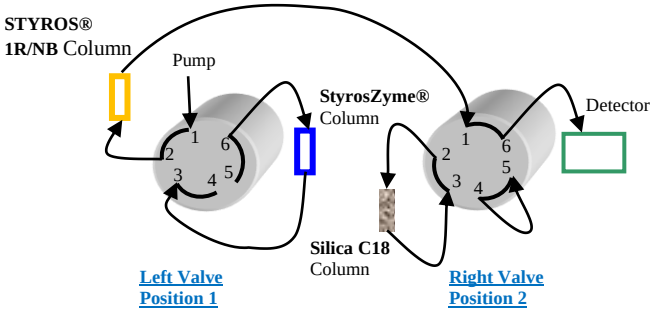
1-Equilibrate the enzyme column with both columns in line as shown in Setup 1.

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 Trypsin sample is injected, and the resulting digests are dumped on the polymeric reversed phase column using the following method:

Time	% of buffer C	Flow rate (ml/min)
0		0
0.01	100	0.1
10	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 in line to equilibrate it with the starting solvent gradient.



Setup 2

3-The reversed phase polymeric 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.

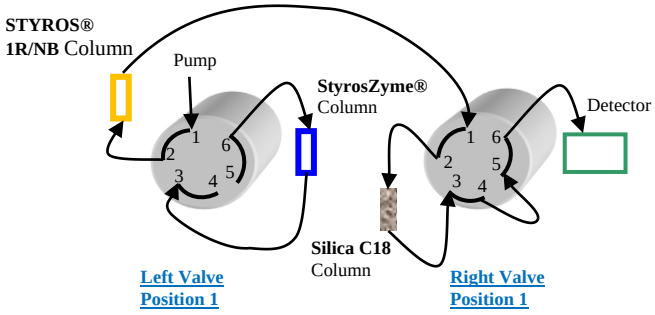
Time	% of buffer B	% of buffer A	Flow rate (ml/min)
0			0
0.01	0	100	0.2
20	60	40	0.2

Once the conditions of the digestion are satisfactory, one

can use the second switching valve to bring the Silica C18 column online 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 in addition.

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
20	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 now washed and 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	60	40	0.3
2	100	100	0.3
4	0	100	0.3
8	0	100	0.3

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.

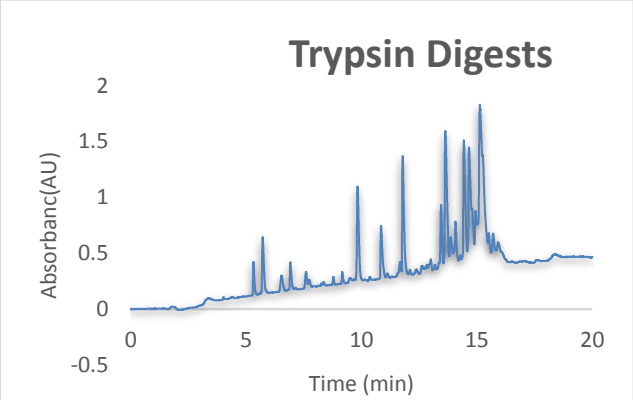
Time	% of digestion buffer C	Flow rate (ml/min)
0		0
0.01	100	0.3
10	100	0.3

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

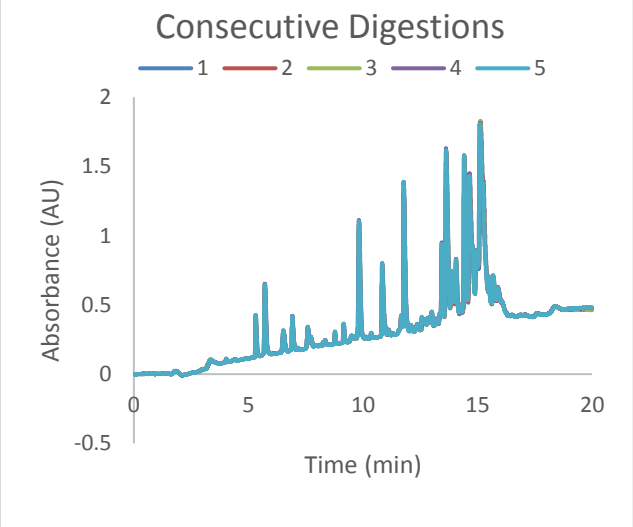
The use of Narrow Bore columns requires minimal use of solvents therefore the automated digestion and mapping can be run around the clock without any concern of running out of buffers or overfilling the waste container.

We have used 5 µl of a solution of 10 mg/ml of Trypsin from bovine as sample to digest.
The temperature is set at 37°C for all sequences.
The digestion is complete, and reproducible with no sign of deactivation of the column's immobilized Trypsin.

The following chromatogram depicts the peptides resulting from the digestion of Trypsin or the auto-digestion to expect during the batch mode digestion processes.
The auto digestion is also expected using immobilized enzyme on leaching media.



Compared with the injected amount of enzyme the digestion is complete.



The enzyme column retains its full enzymatic activity after being exposed to itself.
it is reasonable to conclude that the immobilized Trypsin is restricted in exposing its digestible sites to the free moving Trypsin in solution.

Inc.