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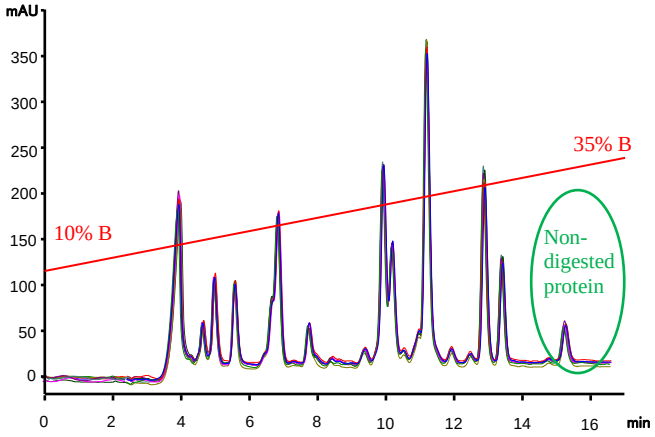
APPLICATION NOTE

Automated Digestion with StyrosZyme® TPCK-Trypsin, Immobilized Enzyme on Polymeric Hard Gel Simulated-Monolith™.
Stability of the Enzyme Reactor in Yielding Reproducible Results in Automation.

In previous Application Notes we have provided the details of the automated digestion using the StyrosZyme® TPCK-Trypsin as the enzyme reactor, followed by STYROS® 2R to trap and map the resulting digests.
We have also checked several variables to verify that the process of digestion remains constant.
In the present Application Note the stability of the StyrosZyme® TPCK-Trypsin is tested for reproducibility as the goal of the automation is to run the digestion and mapping continuously and under control.



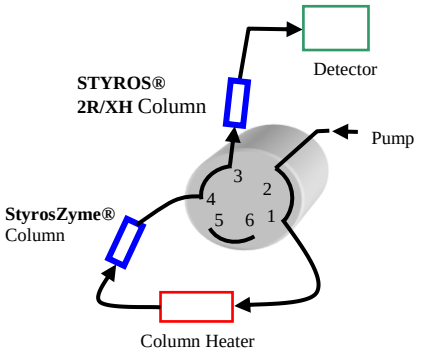
In the above chromatogram multiple automated runs were superimposed to compare variability.
The amounts of non-digested protein remain the same as well as the resulting peptides.
It is to be noted that the column was previously run for more than 500 cycles, that is more runs required for automation around the clock.
Cytochrome c in the present case is injected in its native form and has not been treated as it is the case with digestion in solution.
Trypsin/TPCK-Trypsin is the most used enzyme in proteomic research. It has therefore been the focus of our first set of Application Notes for automation.
The support on which the enzymes are covalently tethered are stable polystyrene cross-linked with divinyl benzene.
They are made as Simulated-Monolith™ to have fully convective and non-restrictive flow path.
The pore size restrictions are no longer an issue either. The media can accommodate large proteins as well as small peptides.
The enzyme reactor can tolerate pressures of more than 5,000 PSI without any negative impact on its activity.

The operating parameters remain the same as in Application Notes 132 and 133:

HPLC System.	Agilent 1100 with thermostatted column compartment and a 6-port valve.
Columns	**StyrosZyme® TPCK-Trypsin** 2.1 X 100 mm **STYROS® 2R/XH** 4.6 X 150 mm
Mobile phase.	A: 0.075 % TFA in DI H2O B: 0.075 % TFA in Acetonitrile: H2O 95:5 C: 0.1 M Tris, pH=8.5
Flow rates	As indicated in various steps
Gradient	10 to 35 % B in 17 minutes for mapping of resulting peptides.
Temperature	37°C
Detection	214 nm
Injection volumes	10 µl
Sample:	3 mg/ml of Cytochrome c in buffer A.

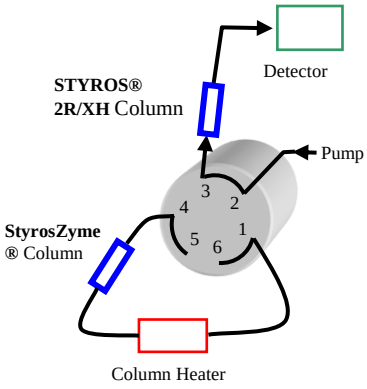
A similar set up is also used as in previous application notes to run the automation.

Setup 1



In the second position, the enzyme column is removed from the line and only the reversed phase STYROS® 2R column remains to map the resulting peptides.

Setup 2



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