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Application Note 521

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Automated 2D LC Coupled to ESI-MS/MS for the Analysis of Complex Peptide Samples

INTRODUCTION

After completing a large number of genome sequences during the last decade attention is now focused on the structure and function of proteins in biological systems.

For more than two decades, commercial 2D electrophoretic techniques have provided the highest resolving power available. Large-scale protein profiling is generally performed by two-dimensional gel electrophoresis (2D GE) followed by MS or LC-MS.

Nano LC run is between 50 and 75 peaks. Although modern MS instruments can fractionate multiple components simultaneously, still the cycle time is limiting the amount of information when too many peaks are co-eluting. Therefore, the analysis of very complex samples requires more resolving power of the chromatographic system. In recent years, the advantages of two-dimensional methods based on liquid chromatography have become evident.

RESULTS AND DISCUSSION

In this application the FAMOS micro autosampler, the UltiMate Nano LC system and the Switchos microcolumn switching unit are used for 2D LC analysis of a complex peptide mixture. The method is based on fractionation of peptides on a strong cation exchange stationary phase and separation on a reversed phase Nano LC column.

Five standard proteins, cytochrome c, alcohol dehydrogenase, bovine serum albumin, apo-transferrin and B-galactosidase were reduced with dithiotreitol and carboxymethylated prior to digestion with trypsin. The

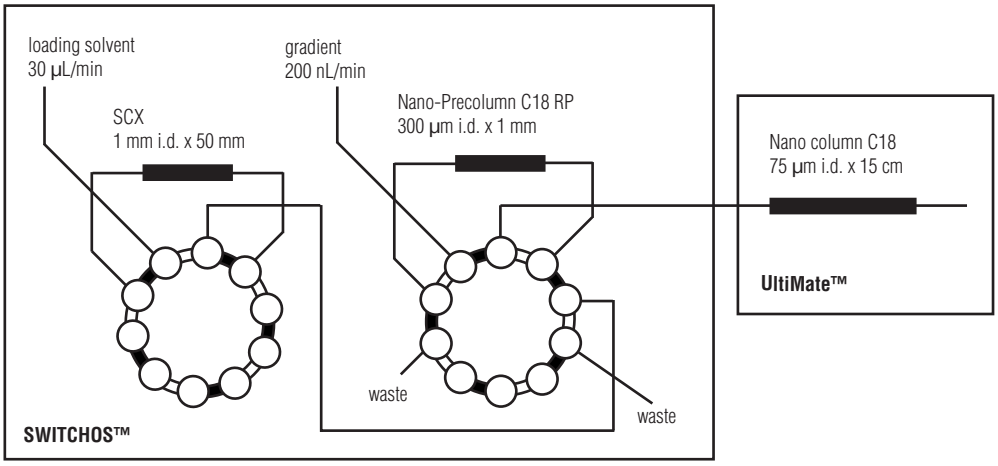

Figure 1. Experimental set-up for 2D LC.

digested sample contained several hundred peptides which could not be separated in a single chromatographic run as shown in Figure 2 the upper chromatogram.

In the 2D LC method the same sample was injected onto a 1 x 15 mm SCX column. During sample injection a reversed phase precolumn of 300 µm x 1 mm is in-line with the SCX column. All non-binding peptides are retained on the reversed phase trapping column. Next the reversed phase precolumn is switched in-line with minutes are shown for the single run and the 25 mM fraction. In the single run several peptides co-elute, whereas for the 25 mM fraction the signal is mainly from the 700 m/z peptide. The increase in chromatographic resolution is at the cost of analysis time. In this example the analysis time is increased by a factor of 7, since the sample was fractionated in 7 steps. Depending on the complexity of the sample more or less fractions can be performed.

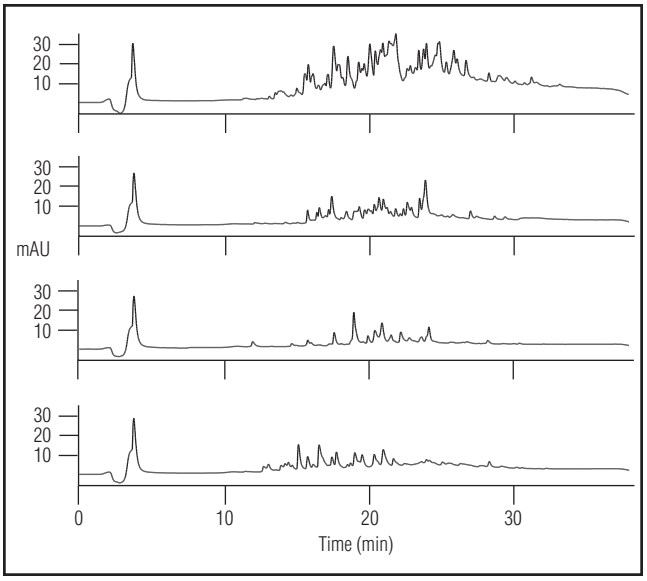

Figure 2. Chromatograms of the digest mixture. Upper trace is the result of a separation without SCX. The next chromatograms are the result of the 5, 50 and 100 mM fractions. (Not all fractions are shown).

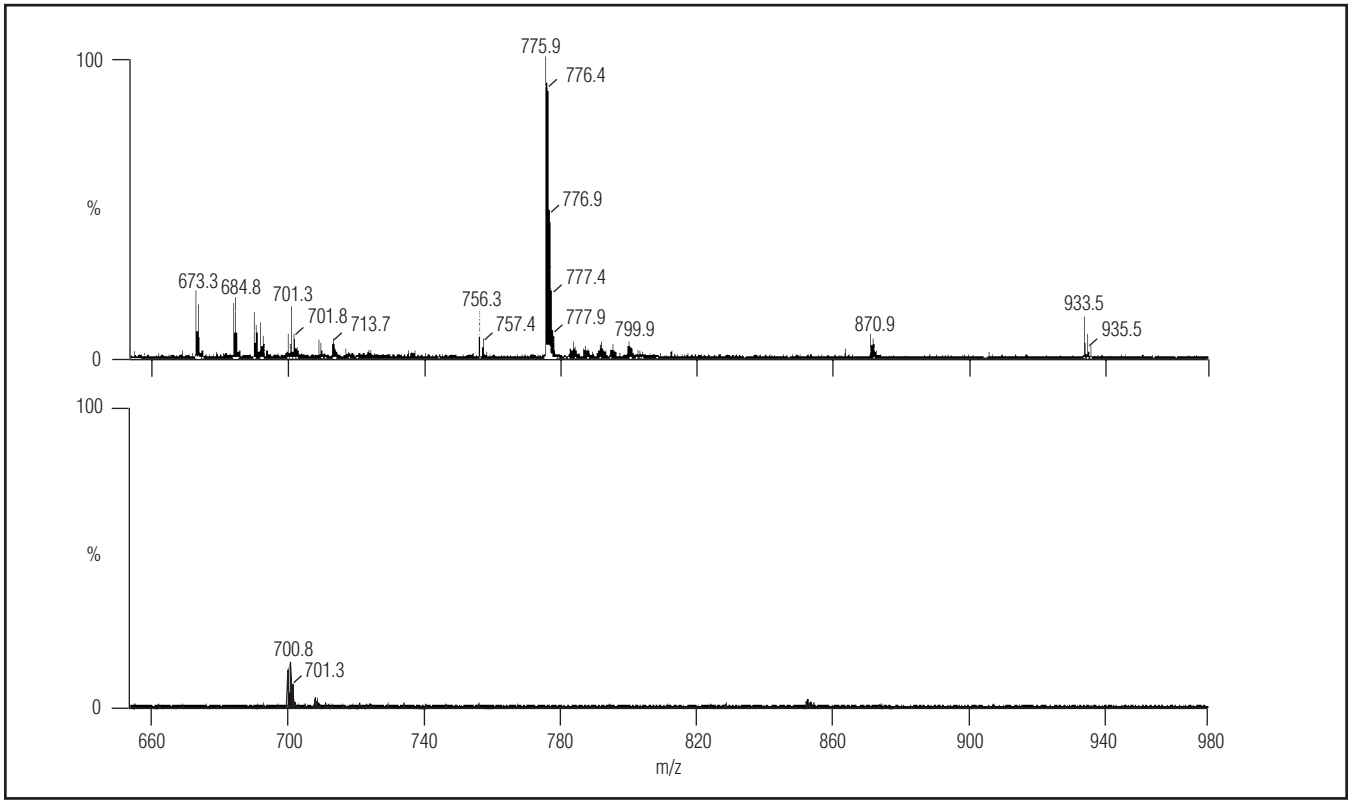

Figure 3. MS scan at 38 minutes in the chromatograms of the single run and 25 mM fraction respectively.