

UltiMate™ 3000 — Direct Sample Injection onto a 75- μm i.d. PepMap™ 100 (C18) Nanocolumn

INTRODUCTION

Reversed-Phase High Pressure Liquid Chromatography (RP-HPLC) combined with tandem MS detection is the main tool for high throughput peptide sequencing. The selection of the chromatographic system depends on the amount of sample, the complexity, and the presence of salts. Samples that consist of less than one hundred peptides and that are free of salts can be directly injected onto a reversed-phase column.

This type of analysis is often preceded by off-line micropreparative procedures such as sample purification or preconcentration by solvent evaporation.

In this technical note, we describe the UltiMate 3000 setup that allows direct sample injection onto a 75- μm i.d. PepMap 100 (C18) nanocolumn.



EXPERIMENTAL

The fluidic setup for direct injections onto a 75- μm i.d. nanocolumn is shown in Figure 1. The nanocolumn is directly connected to the injection valve. The mounted 1- μL injection loop is flushed through in several minutes at a typical flow rate of 300 nL/min. Tubing from the splitter and from the injection valve is 20- μm i.d. to facilitate fast gradient delivery to the column.

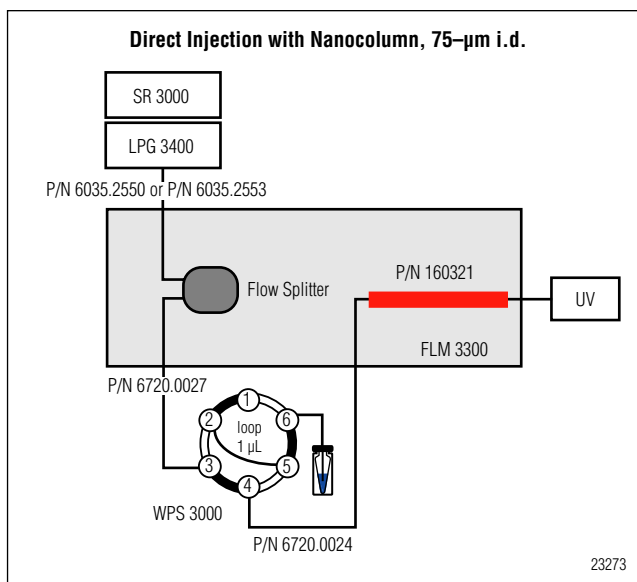


Figure 1. Fluidic connections for a direct injection on a 75- μm i.d. PepMap 100 nanocolumn.

The LC conditions were as follows:

LC system: UltiMate 3000
Column: PepMap 100, C18, 3 μm , 100 \AA ,
75- μm i.d. x 15 cm (P/N 160321)
Mobile Phase Eluent: A) 100% Water, 0.05% TFA
B) 20:80, Water:MeCN, 0.04% TFA
Gradient: 4–55% B in 30 min, 90% B for
5 min, 25 min equilibration
Flow Rate: 300 nL/min
Oven Temperature: 25 $^{\circ}\text{C}$
Sample: Cytochrome c digest, 1 pmol/ μL
Inj. Volume: 1 μL
Detection: UV, 214 nm, 3-nL flow cell
WPS Temperature: 5 $^{\circ}\text{C}$

RESULTS

A typical separation of a cytochrome c tryptic digest sample under ion-pairing RP conditions is shown in Figure 2.

The chromatogram is characterized by ten main peptide signals separated with high resolution and excellent peak shapes. This separation can be used as a tool to validate the UltiMate 3000 Nano LC system. More complex samples might require longer gradient for complete separation. Using these 75- μm i.d. columns and flow rates of around 300 nL/min, peak elution volumes are typically less than 150 nL. Proper zero dead volume connections are of utmost importance under these conditions.

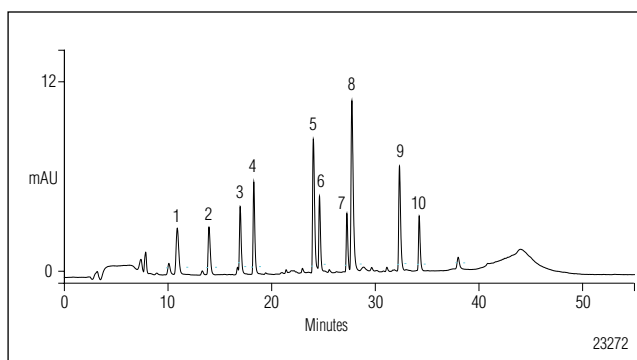


Figure 2. Typical chromatogram for a cytochrome c digest separated on a 75- μm i.d. PepMap 100 nanocolumn.

CONCLUSIONS

The UltiMate 3000 system allows for high-resolution peptide mapping in direct sample injection mode. This method is applicable to low to medium complex samples that do not require desalting and/or preconcentration. The separation of a simple peptide mixture (e.g., a cytochrome c digest) can be used to validate the system.

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