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.

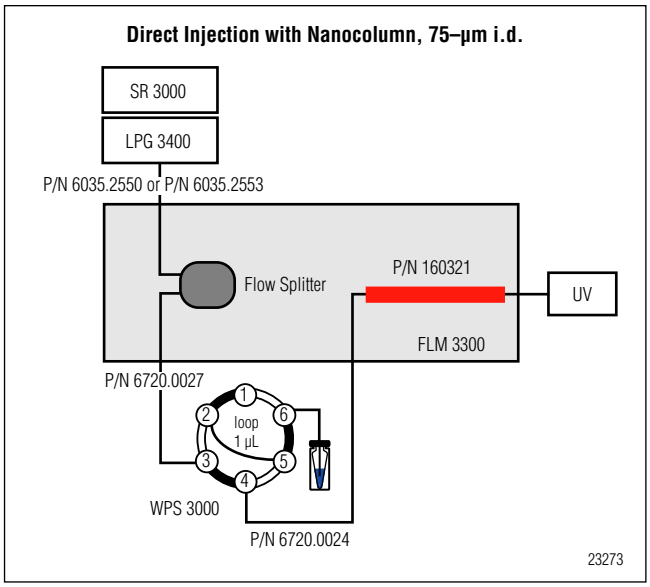

Figure 1. Fluidic connections for a direct injection on a 75-µm i.d. PepMap 100 nanocolumn.

The LC conditions were as follows:

LC system:	UltiMate 3000
Column:	PepMap 100, C18, 3 µm, 100 Å, 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 °C
Sample:	Cytochrome c digest, 1 pmol/µL
Inj. Volume:	1 µL
Detection:	UV, 214 nm, 3-nL flow cell
WPS Temperature:	5 °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.

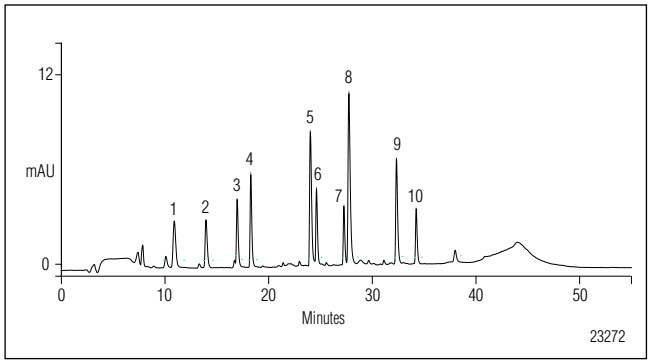

Figure 2. Typical chromatogram for a cytochrome c digest separated on a 75-µm i.d. PepMap 100 nanocolumn.

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.

Dionex Corporation
1228 Titan Way
P.O. Box 3603
Sunnyvale, CA
94088-3603
(408) 737-0700

North America
U.S. (847) 295-7500
Canada (905) 844-9650

South America
Brazil (55) 11 3731 5140

Europe
Austria (43) 1 616 51 25 Belgium (32) 3 353 4294 Denmark (45) 36 36 90 90
France (33) 1 39 30 01 10 Germany (49) 6126 991 0 Italy (39) 02 51 62 1267
The Netherlands (31) 20 683 9768 Switzerland (41) 62 205 9966
U.K. and Ireland (44) 1276 691722

Asia Pacific
Australia (61) 2 9420 5233 China (852) 2428 3282 India (91) 22 28475235
Japan (81) 6 6885 1213 Korea (82) 2 2653 2580 Singapore (65) 6289 1190

www.dionex.com

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