

UltiMate™ 3000 — Preconcentration on a 75- μm i.d. x 15 cm PepMap™ 100 (C18) Nanocolumn

INTRODUCTION

A strategy commonly used for the enrichment of trace components from complex mixtures in liquid chromatography is to preconcentrate the sample on a trap column prior to separation (i.e., when large volume injections need to be performed). This technique can also be applied for the desalting of compound mixtures isolated from biological matrices that often contain high amounts of nonvolatile buffer salts. The presence of such buffers may interfere with the operation of electrospray ion sources by suppressing ionization. In this technical note, the UltiMate 3000 setup for preconcentration and desalting of samples is described.

EXPERIMENTAL

A preconcentration LC experiment was carried out on the UltiMate 3000 system, using PepMap 100 (C18) nanocolumns for peptide trapping and separation. The C18 trap and separation columns were mounted in the FLM module of the UltiMate 3000 in a column-switching setup, as illustrated in Figure 1. The sample solution was loaded onto the trap column at a flow rate of 30 $\mu\text{L}/\text{min}$. After loading, the sample components were eluted from the trap column in a counter flow direction with a linear gradient and separated on the nanocolumn. The method was applied for a cytochrome c tryptic digest.

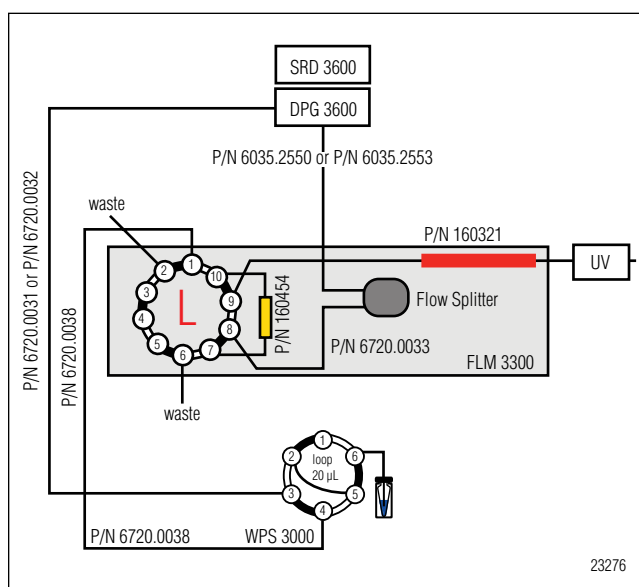


Figure 1. Fluidic connections for a preconcentration experiment on a PepMap 100 (C18) nano column.

The LC conditions were as follows:

LC system:	UltiMate 3000
Separation Column:	PepMap 100, C18, 3 μm , 100 \AA , 75- μm i.d. x 15 cm (P/N 1603211)
Eluent Mobile Phase:	A) Water, 0.05% TFA B) 20:80, Water:MeCN, 0.04% TFA
Gradient:	4% to 55% B in 30 min, 90% B for 5 min, 25 min equilibration
Flow Rate:	300 nL/min
Trap Column:	PepMap 100, C18, 5 μm , 100 \AA , 300- μm i.d. x 5 mm,
Loading Solvent:	98:2, Water:MeCN, 0.05% TFA
Loading Flow:	30 $\mu\text{L}/\text{min}$
Loading Time:	3 min
Sample:	Cytochrome c digest, 100 fmol/ μL
Inj. Volume:	10 μL
Detection:	UV, 214 nm
Oven Temperature:	25 $^{\circ}\text{C}$
Sample Cooling:	5 $^{\circ}\text{C}$

RESULTS

A typical separation performed on a preconcentration LC setup is shown in Figure 2.

Peptides are concentrated on the 300- μm i.d. PepMap 100 (C18) trap column using a second loading pump that facilitates the injection of large volumes in nano LC. In addition, on-line desalting of samples can be realized.

The separation on the 75- μm i.d. PepMap 100 (C18) nanocolumn in Figure 2 demonstrates the high chromatographic resolution. Using the preconcentration setup, the chromatographic resolution is identical to that obtained with the direct sample injection setup.¹

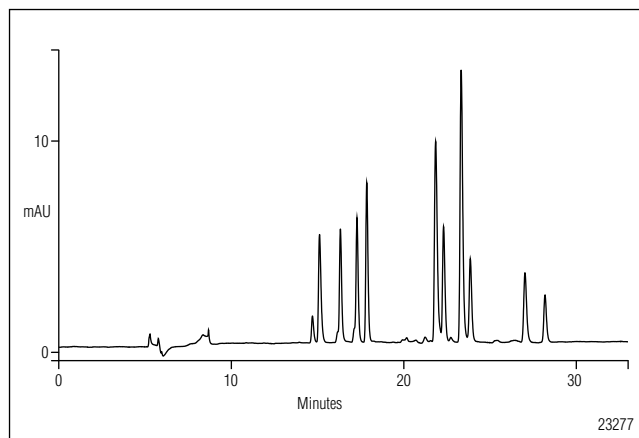


Figure 2. Typical chromatogram for a preconcentration experiment on a PepMap 100 (C18) nanocolumn.

CONCLUSIONS

The UltiMate 3000 system in preconcentration mode forms an ideal instrument for the preconcentration and on-line desalting of samples. The system combines high chromatographic resolution separations and flexibility towards sample injection. In addition, with minor changes the system can be extended to a 2-D LC system.

REFERENCES

1. UltiMate 3000—Direct Sample Injection onto a 75- μm i.d. PepMap 100 (C18) Nanocolumn. Technical Note 60, LPN 1869, in press. Dionex Corporation, Sunnyvale, CA.

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