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## The UltiMate 3000 MudPit Solution

### INTRODUCTION

One of the most common LC-MS applications in proteomics is the multidimensional protein identification technology, or the MudPit approach. With this technique, complex tryptic digests are separated first on a strong cation exchange (SCX) column, and then on a reversed-phase (RP) column. Typically, both columns are combined and directly coupled to a mass spectrometer.<sup>1</sup>

In the MudPit approach, a quaternary gradient pump is used for the RP gradient (AB), and to provide the salt (CD) to elute consecutive fractions from the column. The nano columns are typically loaded off-line, and then connected to the system to perform the 2D analysis. The analysis can take up to 24 h, given that the columns must be disconnected from the system, reloaded, and then reconnected each day.

In this Technical Note (TN), the authors demonstrate the MudPit application based on the UltiMate<sup>®</sup> 3000 platform. In addition, features to automate sample introduction are demonstrated that minimize the number of reconnections. This ultimately improves column lifetime.

### EXPERIMENTAL

The SCX and RP columns in MudPit are always in-line with each other; they can be joined together with a zero-dead volume union or one column can be packed with the two stationary phases (biphasic column). The sample is loaded on the first column, and then a series of nanoflow gradients is run with the SCX elution plug, increasing in strength.

MudPit analysis requires three mobile phases: the two for the RP gradient (AB) and one for the SCX elution (C). An SCX wash solution is commonly used as the fourth eluent (D). The gradient profile for the 50% C elution step is represented schematically in Figure 1.

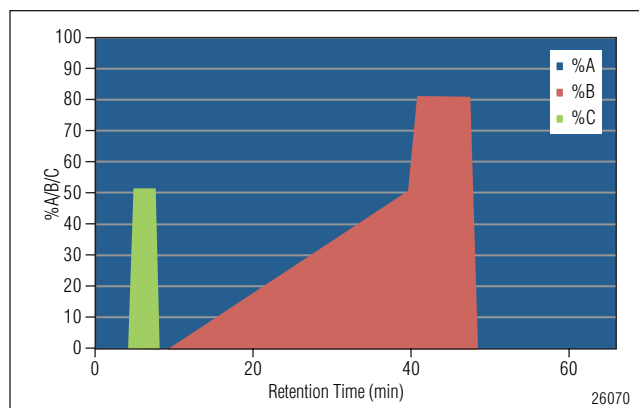


Figure 1. Gradient profile for the 50% SCX elution step. In consecutive runs, the concentration of C solvent increases to elute the sample from the SCX column.

## System

The nano flow ternary gradients can be formed on an UltiMate 3000 system consisting of a single LPG-3400M quaternary micropump and one FLM-3300 flow manager. The UltiFlow™ eluent delivery system is designed to generate nano flow gradients independent of solvent composition. This ensures chromatographic reproducibility and a more stable electrospray.

In addition to providing the UltiFlow technology, the FLM also functions as a column oven and is equipped with a 10-port, 2-position switching valve. The fluidic configurations shown in Figures 2 and 3 demonstrate how this valve can be used with a manual injection kit to allow sample loading onto the column without disconnecting it. There is no added dead volume; after the injection the valve is switched and remains in that position during the remainder of the analysis.

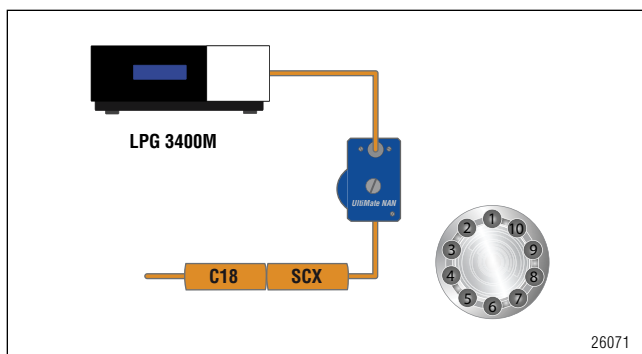


Figure 2. MudPit basic setup.

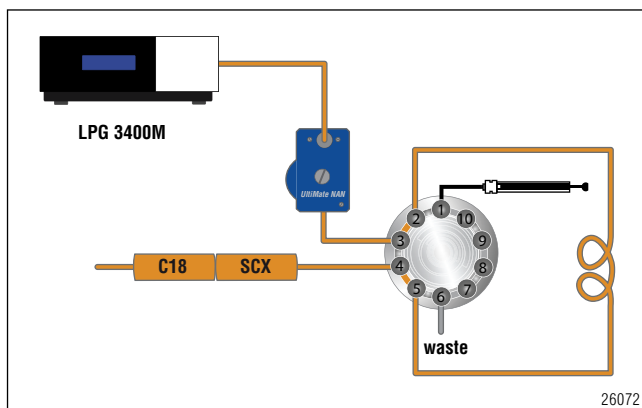


Figure 3. MudPit setup using the 10-port valve with a manual injection kit. There is no extra dead volume when the valve is in position 1-2.

## Chemistry

The two columns described below were connected using a Microtight® union to create the MudPit column:

1. 100  $\mu\text{m}$  i.d.  $\times$  5 cm, packed with 5  $\mu\text{m}$ , BioX-SCX (P/N 161394)
2. 75  $\mu\text{m}$  i.d.  $\times$  15 cm, packed with Acclaim® PepMap™ column, 100 C18, 3  $\mu\text{m}$ , 100Å (P/N 160321)

The solvents used with the quaternary pump are listed below. All were LC-MS grade quality:

- Mob A: 98/2 H<sub>2</sub>O ACN 0.05% FA  
 Mob B: 20/80 H<sub>2</sub>O ACN 0.04% FA  
 Mob C: Mob A + 250 mM NH<sub>4</sub>Ac  
 Mob D: Mob A + 500 mM NH<sub>4</sub>Ac

The method was tested with tryptic digests of a six-protein mix (PMD) and an *E.coli* cell lysate.

Table 1. Gradient Example for the 50% C Program			
Time (min)	% A	% B	% C/D
0	100		
0.5	100		
1	50		50*
3	50		50*
3.5	100		
6	100		
36	50	50	
37	10	90	
42	10	90	
43	100		
66	100		

\*In consecutive programs, this percentage was 0%, 2.5%, 5.0%, 7.5%, 10%, 20%, 30%, 40%, 50%, and 100% C followed by a wash of a 100% D.

## RESULTS

All six proteins were identified in the PMD sample with a sequence coverage of more than 60% for the five proteins up to 100 kDa. The larger beta galactosidase (114 kDa) was identified with 38% sequence coverage.

The MS data for the *E. coli* samples are shown in Figure 4. The data consists of base peak chromatograms (BPC) of the increasing salt elution steps (indicated as the % C). The RP gradients are identical in all runs. For further details see Figure 1 and Table 1.

In the *E. coli* sample, 366 proteins were identified confidently by at least two peptides that had an ion score >15.

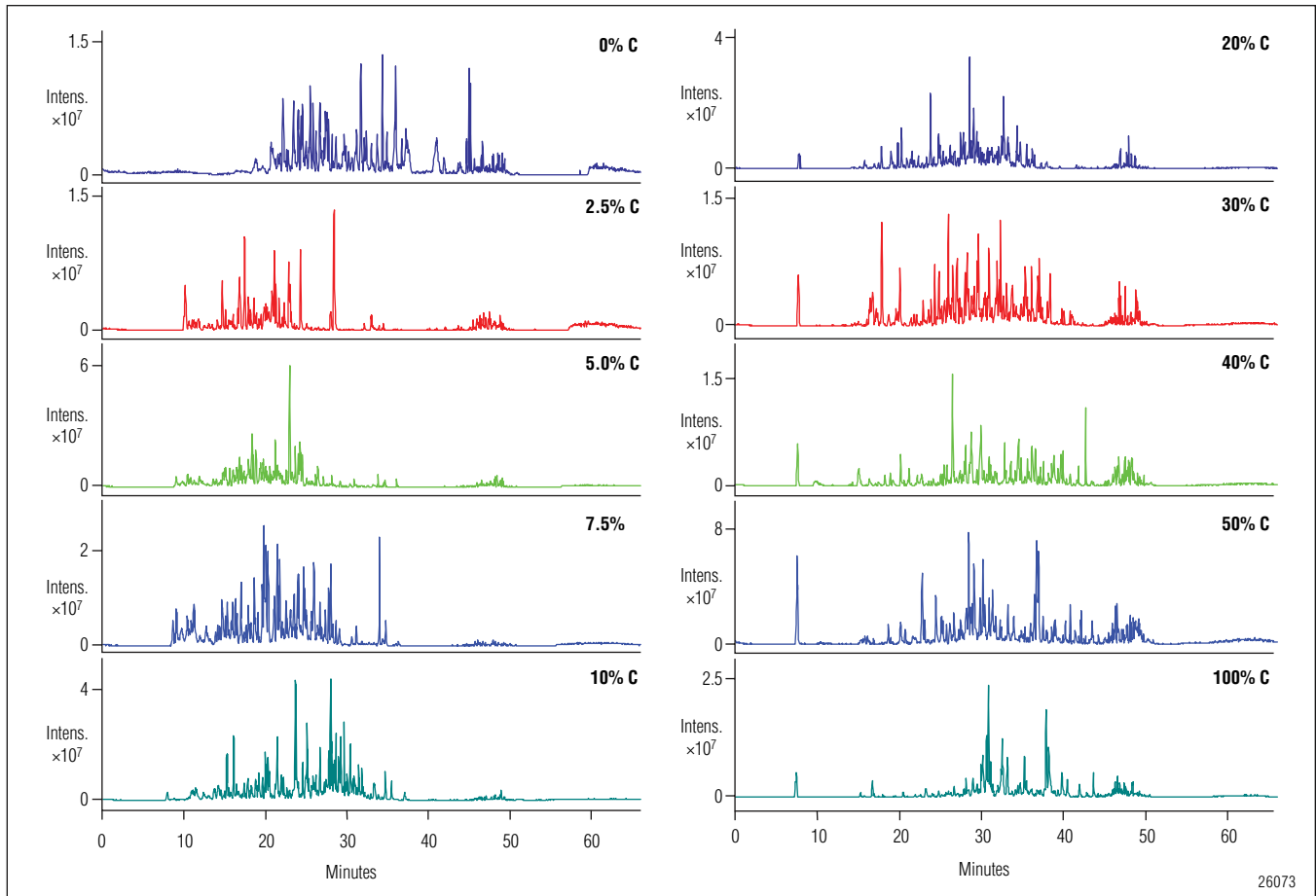
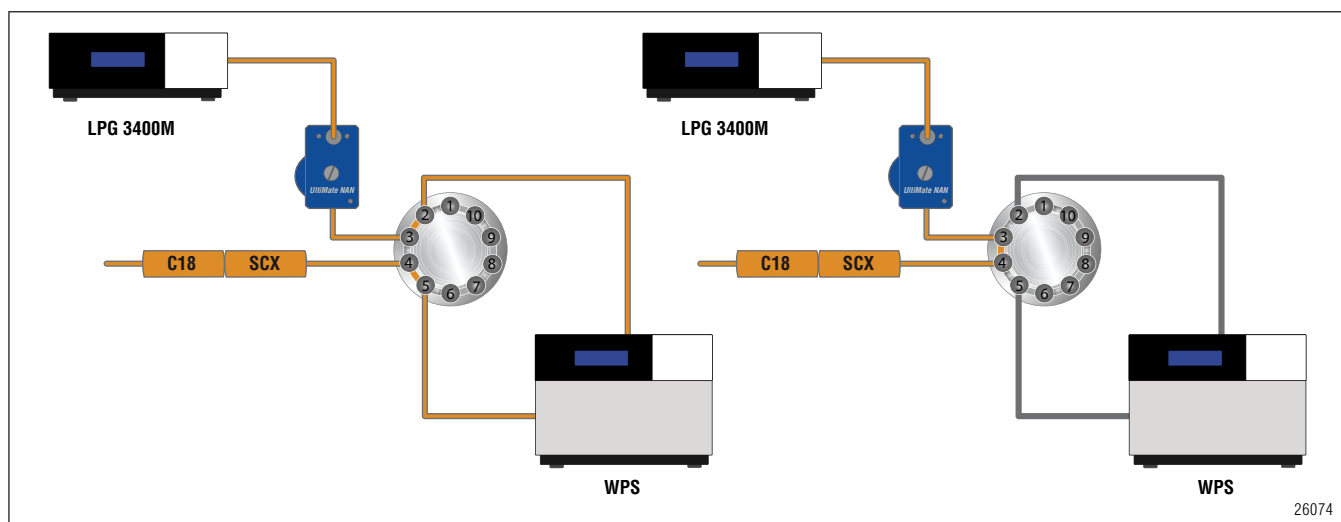


Figure 4. BPC of the MudPit separation of the *E. coli* cell lysate digest.



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Figure 5. Application of the WPS in the MudPit approach. Left: Injection of sample. Right: Autosampler bypassed during analysis.

### FULL AUTOMATION

The UltiMate 3000 MudPit solution can easily be expanded with a WPS Nano autosampler to run multiple samples automatically (see Figure 5). Here, a dedicated inject program switches the autosampler in-line to transfer the sample to the column. When the sample is loaded, the autosampler is switched off-line and the sample is analysed.

### CONCLUSIONS

The UltiMate 3000 MudPit solution efficiently combines the established MudPit separation strategy with the flexibility and performance of the UltiMate 3000 nano LC system. The UltiFlow eluent delivery system ensures accurate column flow throughout the ternary gradient.

Expanding the system with a manual or automated injection solution minimizes the need for column reconnections and improves method durability. In addition, automated injection allows unattended performance of multiple, consecutive analyses.

### REFERENCES

1. Yates *et al.*, Large-Scale Analysis of the Yeast Proteome by Multidimensional Protein Identification Technology, *Nature Biotechnology* **2001**, Vol 9, 242–247.

Materials	
Description	Part Number
LPG 3400M	5035.0045
FLM 3300	5720.0030
Connection tubing 20 µm ID x 30 cm	6720.0033
100 µm i.d. x 5 cm, packed with 5 µm, BioX-SCX resin	161394
75 µm i.d. x 15 cm, packed with Acclaim PepMap 100 C18, 3 µm, 100 Å resin	160321
µ Tight union	161497
Optional Injection	
Manual injection kit	Contact local sales
Automated Injection	
WPS-3000 PL	5820.0010
2 x connection tubing 20 µm i.d. x 50 cm	6720.0036

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