

Parallel Nano and Capillary LC for High-Throughput MS Proteomics

INTRODUCTION

A new UltiMate™ Plus Dual-Gradient Nano and Capillary LC System has been developed that allows for parallel capillary/nano LC separations. Throughput in nano and capillary LC-MS using this system can be increased substantially by analysing a sample on one column while equilibrating the second column. A typical nano LC separation consists of a 30-min gradient, with 10–15 min for equilibration and 0–5 min for sample loading. Sample components (e.g., peptides) usually elute during the 10–50% acetonitrile portion of the gradient.

Only 40–50% of the total analysis time is relevant for the identification of peptides by MS. Using the UltiMate Plus Dual-Gradient System, the equilibration time can be used for loading and running a second sample, and the MS acquisition time is only used during the relevant part of the sample. Consequently, the MS throughput can increase by 30–40%.

In this application note, we present the fluidics of such a parallel nano and capillary LC system and demonstrate a significant gain in MS throughput.

EXPERIMENTAL

Figure 1 shows the experimental setup. Two 10-port switching valves are used to connect the trapping columns and nano LC separation columns. An ultralow dead volume 6-port switching valve is used to select between the two separation columns and guide the effluent to the MS with virtually zero chromatographic dispersion.

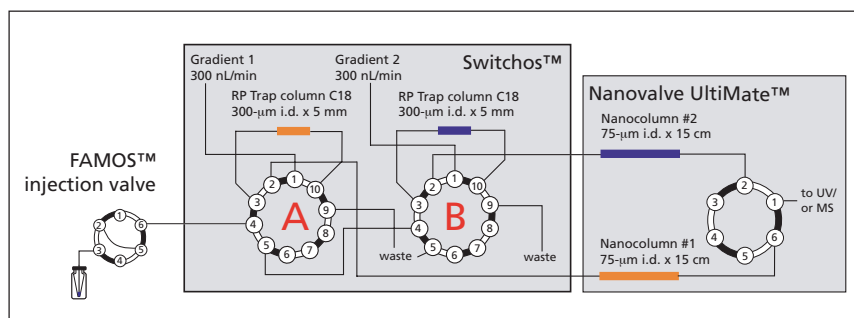


Figure 1. Fluidic connections for parallel nano LC.

Nano LC System

LC system:	UltiMate Plus Dual-Gradient (P/N 163644)
Autosampler:	FAMOS Well Plate (P/N 163655)
Column switching unit:	Switchos (P/N 163662)
Postcolumn valve:	Nanoswitching Valve (P/N 161734)
MS:	esquire3000 plus, nanospray source (Bruker Daltonics)

Columns and Mobile Phases

Columns 1 and 2: PepMap™ C18, 75- μm i.d. \times 15 cm, 3 μm , 100 \AA (P/N 160321)

Mobile phase A: 0.1% formic acid in water

Mobile phase B: 0.08% formic acid in water-ACN (20:80, v/v%)

Gradient: 0% B to 40% B in 30 min; hold 5 min at 90% B

Flow rate: 300 nL/min

Trap columns 1 and 2: PepMap C18, 300- μm i.d. \times 5 mm, 3 μm , 100 \AA (P/N 160454)

Loading mobile phase: 0.1% TFA in water

Loading flow rate: 30 $\mu\text{L}/\text{min}$

Detection Conditions

UV: 214 nm

MS capillary voltage: 1200 V

Spray needle: 20- μm i.d. (New Objective)

Scan range: 450–2000 m/z

Sample

Cytochrome c tryptic digest: 500 fmol/ μL (P/N 161089)

BSA tryptic digest: 300 fmol/ μL

Protein mixture digest: 1.0 pmol/ μL (P/N 161088)

Injection volume: 1 μL

Loading mobile phase: 0.1% TFA in water

A typical HPLC solvent gradient consists of the (linear) gradient, column wash, and equilibration step. In the parallel nano and capillary LC mode, the solvent gradient on pump 1 runs simultaneously with the column wash and equilibration step on pump 2. Samples are injected, alternating on trap column 1 and 2.

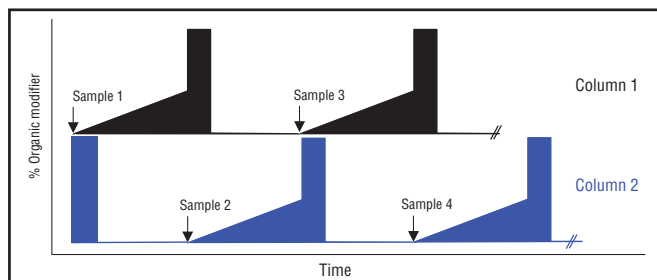


Figure 2. Sample injection and gradient profiles for the parallel capillary/nano LC system, illustrating overlapped injections.

RESULTS

Figure 3 shows consecutive nano LC separations of various tryptic digest samples. A solvent gradient from 0–40% B was programmed in 30 min. The base peak chromatograms (BPCs) clearly show the elution of peptides over the course of the gradient. In parallel LC mode, analyzing a sample on the second LC column while the first is being washed and equilibrated results in optimal use of the MS and significantly higher throughput.

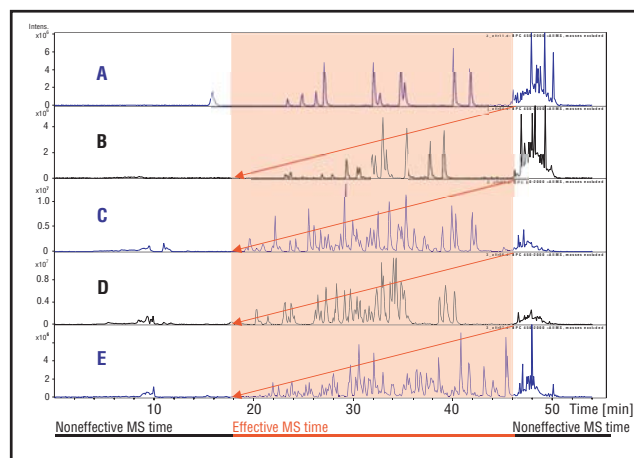


Figure 3. Consecutive nano LC separations of tryptic digest samples of (A) cytochrome c, (B) alcohol dehydrogenase, (C) bovine serum albumin, (D) apo-transferin, and (E) β -galactosidase respectively. Injected amount 500 fmol. Signal is BPC MS trace 450–2000 m/z .

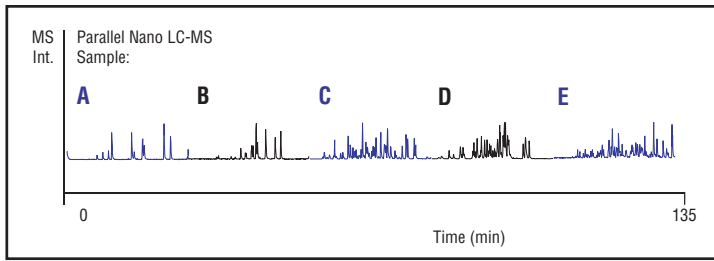


Figure 4. Increased sample throughput with parallel nano LC-MS of 100%.

The total analysis time of the consecutive nano LC separations of Figure 3 is 270 min. Using the parallel nano LC system, the analysis time can be reduced by a factor of 2, as shown in Figure 4.

Reproducibility of the retention times is an important criteria in HPLC. In particular for the parallel nano LC system, where at fixed time points the nanovalve is switched to introduce the peptides into the MS. In Figure 5 A and B, consecutive injections of complex protein digest samples are shown. The obtained elution profiles show sufficient reproducibility for such types of samples.

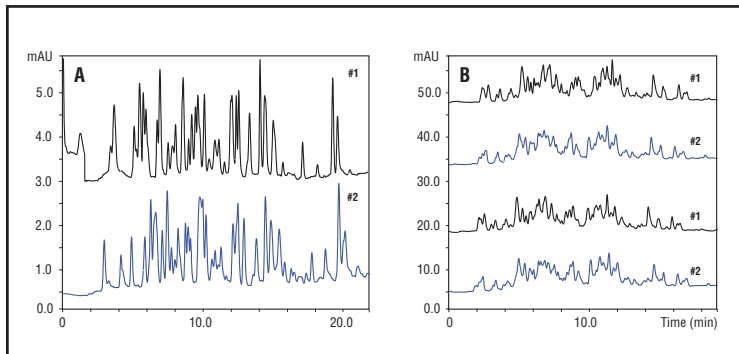


Figure 5: Reproducibility of parallel nano LC; alternated separations on system 1 and 2.

- (A) Consecutive injections of a tryptic digest of BSA. Injected amount 300 fmol, detection UV 214 nm
 (B) Consecutive injections of a protein mixture digest. Injected amount 1 pmol, detection UV 214 nm

CONCLUSIONS

Parallel nano and capillary LC is an efficient method for increasing the sample throughput in MS analysis. Depending on the gradient conditions, the MS throughput can be increased significantly. In our example, an increase in MS throughput of 100% was obtained. With longer gradients, the increase is 30–40%.

Considering the costs of high-end MS instruments and the tremendous increase in MS throughput by using a parallel nano and capillary LC system, substantial cost savings can be achieved.



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* Designed, developed, and manufactured under an NSAI registered ISO 9001 Quality System.

