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Tandem LC approach for LC-MS proteome profiling with near 100% MS utilization

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Summary

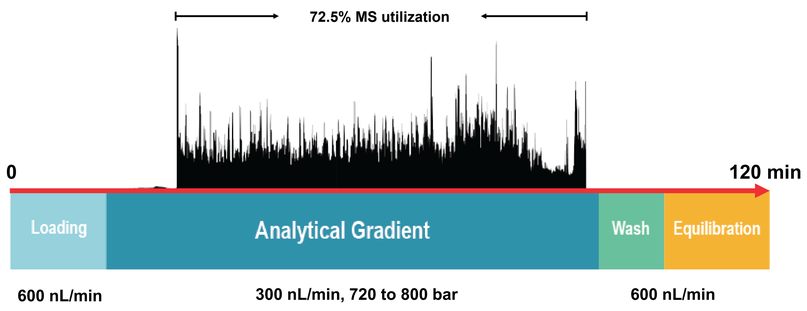
We have developed a novel tandem direct injection workflow on the Vanquish Neo UHPLC system enabling near 100% MS utilization for high-sensitivity and high-throughput proteome profiling in nano and capillary-flow regimes.

INTRODUCTION

Nano/capillary-flow UHPLC coupled with high-resolution accurate-mass (HRAM) mass spectrometry (MS) is the gold standard for deep and quantitative profiling of complex proteomes in discovery proteomics. The high sensitivity of nano/capillary LC-MS, however, is often linked to relatively low MS utilization (i.e., the ratio of peptide elution window vs. total run time). The time that is not utilized for the acquisition of useful MS data is needed for sample injection and loading (typically longer duration in direct injection workflows), column washing and equilibration, and the analytes migrating through the analytical column and fluidics to reach the MS interface (**Figure 1**).

Here we describe a novel tandem direct injection workflow in nano/capillary flow regime (<5 µL/min) that eliminates the limitations of traditional single column setups, where samples are separated on two independent analytical columns and where loading, equilibration, washing steps can be done in parallel with peptides separation. The employment of a new double-barrel ESI source enables for simultaneous interfacing of two separation columns with MS without post-column flow-splitting, to maintain the high chromatographic resolution with long columns (**Figure 2 & 3**).

Figure 1. Limited MS utilization in proteomics analysis using a conventional direct injection workflow with nano flow rates (300 nL/min) and long analytical columns (75 µm I.D. x 75 cm, 2 µm C18).



Tandem direct injection workflow configuration

The tandem direct injection workflow for near 100% MS utilization comprises **(Figure 2 & 3)**:

1) Thermo Scientific™ Vanquish Neo UHPLC System;
2) Thermo Scientific™ Vanquish Column Compartment N with a 2p-6p low-dispersion switching valve;
3) Thermo Scientific™ Vanquish Binary Pump N;
4) Double Barrel Column Oven (Sonation GmbH) installed onto the Thermo Scientific™ Nanospray Flex Ion Source;
5) Thermo Fisher Scientific™ Mass Spectrometer;
6) Intelligent method for automated column switching and data acquisition.

The proposed configuration supports the tandem direct injection workflow using Thermo Scientific™ nanoViper™ fingertight fittings for fluidic connections and are optimized for maximum separation performance (**Figure 4**).

Figure 2. Vanquish Neo Tandem Direct Injection workflow coupled with Orbitrap Exploris 480 Mass Spectrometer.

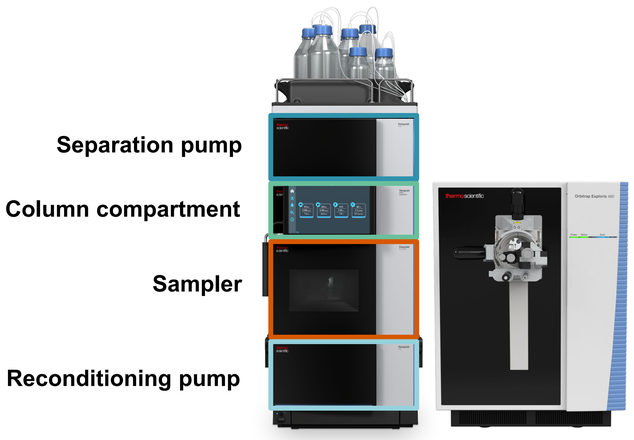
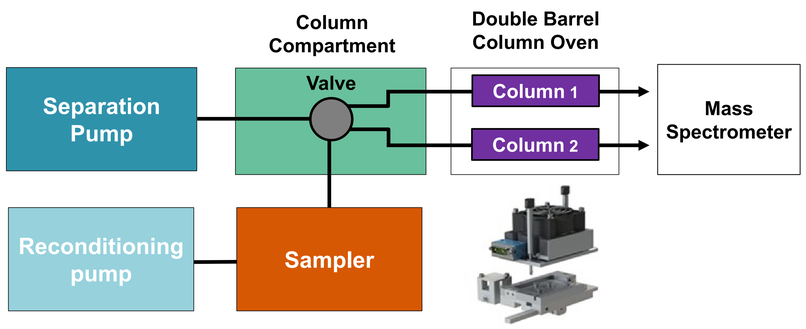


Figure 3. Fluidics connection for tandem direct injection workflow empowered by a double barrel column oven.



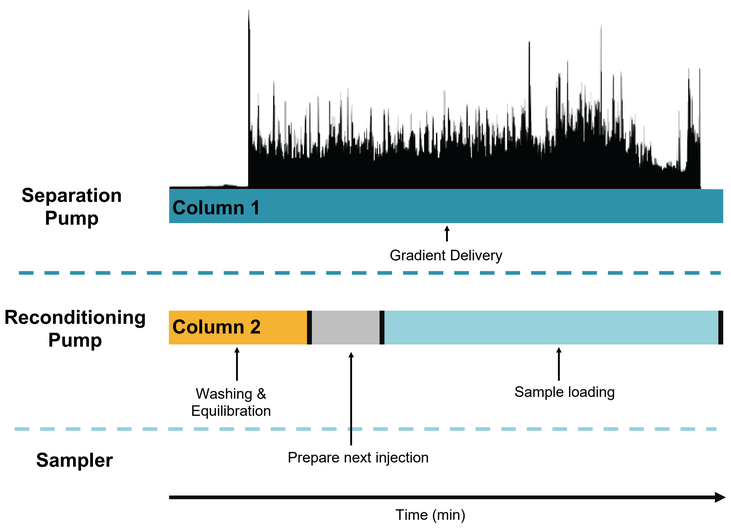
MATERIALS AND METHODS

The lyophilized tryptic peptides were separated using two Thermo Scientific™ PepMap™ Neo UHPLC columns coupled to pulled fused silica ESI emitters (10/30 µm capillary I.D. with 5/10 µm tip I.D., 7 cm in length) with a MicroTight union. The optimized methods incorporated "look-ahead" injections to load the sample onto the second column while the separation on the first column is still on-going and intelligent automated switching between columns to provide a user experience similar to standard LC-MS sequence setup and execution (**Table 1 and Figures 1-4**).

Table 1. Tandem LC-MS/MS method details

	150 µm I.D. x 15 cm	75 µm I.D. x 75 cm
Sample	200 ng PierceTM HeLa Protein Digest Standard	
Mobile Phase A	H2O - 0.1% FA	
Mobile Phase B	80% ACN - 0.1% FA	
Injection Volume	5 µL	
Gradient Flow rate	1.5 µL/min	0.3 µL/min
Temperature	50˚C	
Method Length	8 min	65 min
ESI Voltage Application	Liquid junction on column inlet	
MS Acquisition	DIA & DDA	

Figure 4. The operation principle of the Tandem Direct Injection workflow. The separation pump consistently delivers the gradient for sample elution on column 1 or column 2 while the reconditioning pump and autosampler are used for column washing, equilibration, sample injection and loading

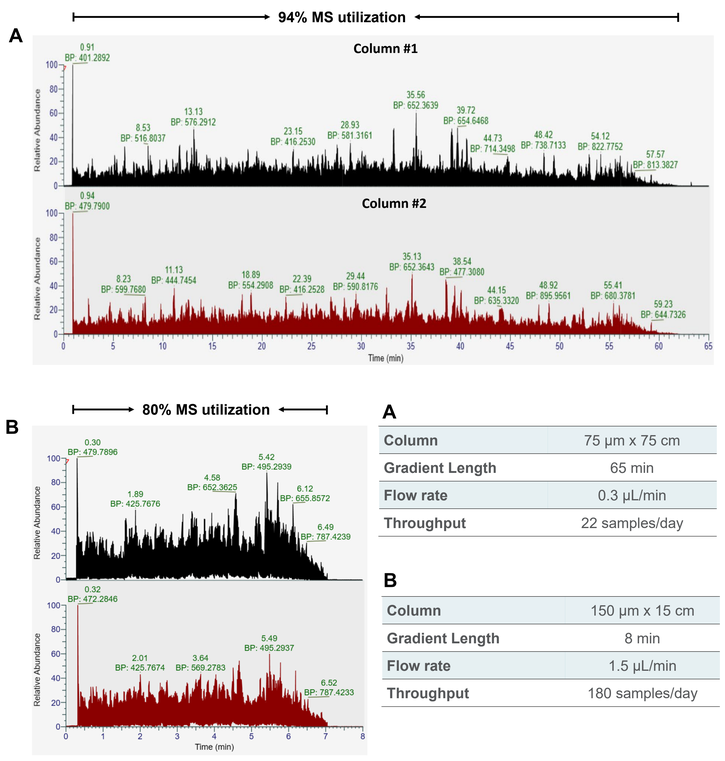


RESULTS

Maximize MS utilization and sample throughput

The tandem direct injection workflow permits ca. 80% and 94% MS utilization from 8- and 65-min gradients, respectively, in 24/7 routine operation for profiling complex protein digests (**Figure 5A and 5B**). A single LC-MS/MS method is required for each flow regime to perform peptide separation and MS acquisition on columns 1 and 2 from run-to-run.

Figure 5. TIC profiles for HeLa peptides separation on column 1 and 2 with 65 min (A) and 8 min (B) methods in tandem direct injection experiments.



A

Column	75 µm x 75 cm
Gradient Length	65 min
Flow rate	0.3 µL/min
Throughput	22 samples/day

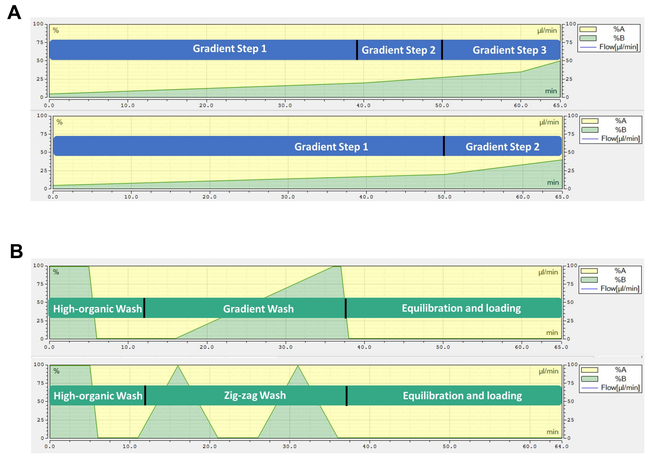
B

Column	150 µm x 15 cm
Gradient Length	8 min
Flow rate	1.5 µL/min
Throughput	180 samples/day

Method versatility of the tandem direct injection workflow

The tandem workflow permits users adjust separation conditions for different sample types (**Figure 6A**), e.g., phosphoproteome and TMT-labeled peptides. Additionally, column washing cycles and equilibration volume can be optimized (**Figure 6B**) to reduce column carryover.

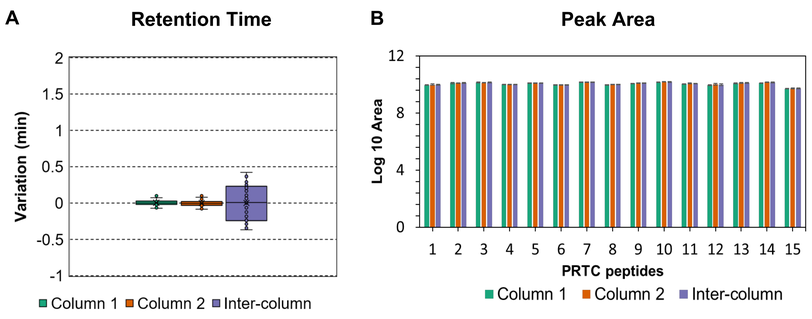
Figure 6. The versatility of defining the gradient for separation (A) and program washing and equilibration cycles (B).



Reproducible intra- and inter-column performance

The tandem workflow provides reproducible chromatographic performance for accurate quantification. For example, we observed <0.5 min retention time variation between two 75 µm x 75 cm columns with a 65-min gradient (**Figure 7A & 7B**), allowing highly reproducible peptide and protein quantification with low-analytical variability.

Figure 7. Excellent nanoLC-MS peptide retention time (A, >0.5 min variation) and peak area (B) reproducibility inter- and intra-column in using the 65 min tandem direct injection method.



CONCLUSIONS

We developed a novel tandem direct injection workflow for maximizing mass spectrometer utilization and demonstrated it's utility using 75 and 150 µm I.D. columns for deep-dive and high-throughput proteome profiling, respectively. This workflow shows high retention time and peak area reproducibility for peptide separations. In addition, the workflow can be seamlessly integrated with all Thermo Scientific mass spectrometers, including the latest Orbitrap Astral mass spectrometer.

The Vanquish Neo tandem direct injection workflow represents a promising alternative to conventional nano/capillary LC-MS setups for shotgun proteomics as well as targeted analysis in complex matrices.

REFERENCES (if necessary)

1. R. Zheng, C. Pynn, etc. New Double Barrel ESI Source and Novel Tandem NanoLC-MS for 24/7 Proteome Profiling with near 100% MS Utilization. TN 73671, Thermo Scientific.
2. A. Boychenko, C. Pynn, etc. High-throughput tandem capillary-flow LC-MS for maximum MS utilization. TN 72827, Thermo Scientific.

TRADEMARKS/LICENSING

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