Novel Tandem Nano and Capillary Flow LCMS-based Approach for Facile 24/7 Proteome Profiling with Near 100% MS

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Summary

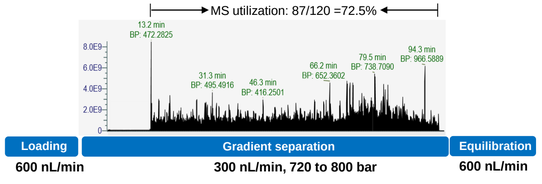
We have developed a novel tandem direct injection workflow on the Vanquish Neo UHPLC system enabling near 100% MS utilization for high-sensitivity and high-throughput proteome profiling in nano and capillary-flow regimes.

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 unmatched 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 workflow), 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 allows for simultaneous interfacing of two separation columns with HRAM-MS without post-column flow-splitting, to maintain the high chromatographic resolution with long columns (**Figure 2** & **3**).

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



Tandem direct injection workflow configuration

The developed tandem workflow allowing routine 24/7 operation with close to 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 Scientific™ Mass Spectrometer;
6) Intelligent method for automated column switching and data acquisition.

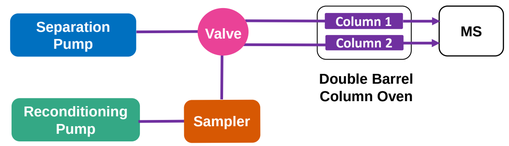
The proposed configuration supports the tandem direct injection workflow using Thermo Scientific™ nanoViper™ Fingertight Fittings for fluidic connections and are optimized for maximum separation performance (**Figure 4**).

Figure 2. Thermo Scientific Vanquish Neo Tandem Direct Injection workflow coupled with Double Barrel Column Oven and Orbitrap Exploris™ 480 Mass Spectrometer

Vanquish Neo UHPLC system (tandem direct injection workflow)–Orbitrap Exploris 480 Mass Spectrometer

Double Barrel Column Oven

Figure 3. Fluidics connection for tandem direct injection workflow



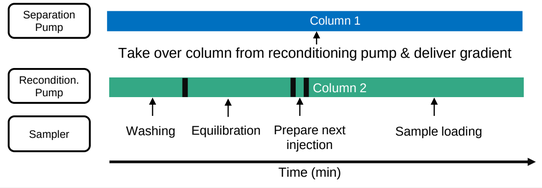
Materials and Methods

The lyophilized tryptic peptides were separated using two separation 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

	PepMap™ 150 µm I.D. x 15 cm	PepMap™ 75 µm I.D. x 75 cm
Sample	200 ng Pierce™ HeLa Protein Digest Standard	
Mobile Phase A	H_2O - 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 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

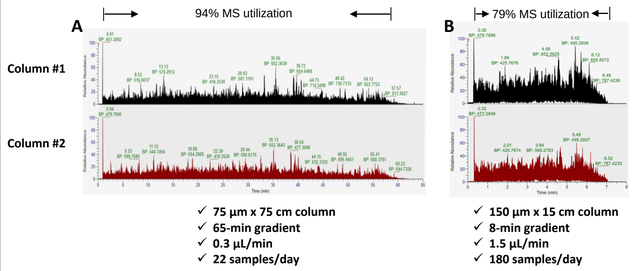


Results

Maximize MS utilization and sample throughput

The tandem direct injection workflow permits 79% 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



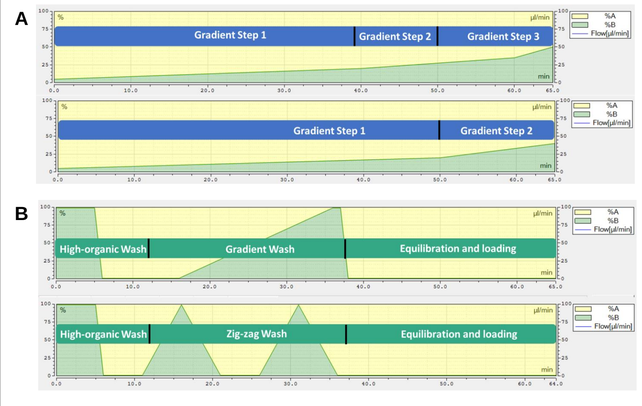
✓ 75 µm x 75 cm column
✓ 65-min gradient
✓ 0.3 µL/min
✓ 22 samples/day

✓ 150 µm x 15 cm column
✓ 8-min gradient
✓ 1.5 µL/min
✓ 180 samples/day

Method versatility of the tandem direct injection workflow

The tandem workflow permits users adjust separation conditions based on the analyzed samples (**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.

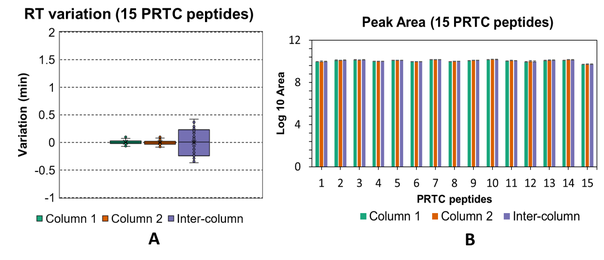
Figure 6. The versatility of defining the gradient for separation (A) and program washing and equilibration cycles (B)



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. An excellent peptide retention time (A, with less than 0.5 min variation) and peak area (B) reproducibility inter- and intra-column in nano-flow LCMS



Conclusions

We developed a novel tandem direct injection workflow that permits maximum MS utilization using 75 and 150 µm I.D. columns for deep-dive and high-throughput proteome profiling. It shows high reproducibility in peptide separation and quantification between columns permitting sample measurement 24/7.

We show how the resolving power of the Vanquish Neo system coupled with HRAM MS and double barrel ESI source can be combined to create a new industry standard in the speed and depth of proteome profiling. This configuration seamlessly integrates with all Thermo Scientific mass spectrometers, including Thermo Scientific™ 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

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.

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