Low-Flow Tandem LC-MS

Novel tandem nano and capillary flow LC-MS-based approach for facile 24/7 proteome profiling with near 100% MS utilization

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Summary

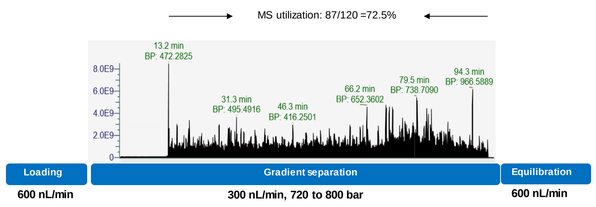
We have developed a novel tandem direct injection workflow on the Thermo Scientific™ Vanquish™ Neo UHPLC system enabling near 100% MS utilization for high-sensitivity and high-throughput proteome profiling at nano and capillary-flow rates.

Introduction

Nano/capillary-flow UHPLC coupled to 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 the time window during which analytes are eluted and analyte date recorded vs. the total run time). The "overhead" time that is not utilized for the acquisition of analyte-based MS data is taken up by critical but time-consuming steps in the analysis cycle. Such steps include sample injection and loading onto the column (which is particularly time intensive in direct injection workflows), column washing and equilibration, along with the time taken for analytes to migrate through the analytical column and fluidics to reach the MS interface (**Figure 1**).

Here we describe a novel tandem direct injection workflow employing a nano/capillary flow regime (≤5 µL/min), that eliminates the limitations of traditional single column setups. Here samples are separated on two independent analytical columns in such a way that the loading, equilibration and washing steps can be done on one column whilst analytes are being separated on the other. The employment of a new double-barrel ESI source permits the 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 a direct injection workflow using a nano flow rate (300 nL/min) and a long analytical column (75 µm I.D. x 75 cm, 2 µm C18).



Tandem direct injection workflow configuration

The tandem workflow presented here, allowing routine 24/7 operation with close to 100% MS utilization, (Figure 2 & 3) comprises:

1. 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.

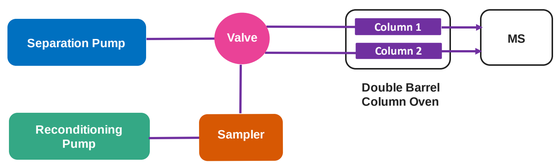
The proposed configuration supports the tandem direct injection workflow using fingertight Thermo Scientific™ nanoViper™ fittings for fluidic connections which are optimized for maximum separation performance (**Figure 4**).

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

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

Double Barrel Column Oven

Figure 3. Fluidic connection scheme for the tandem direct injection workflow



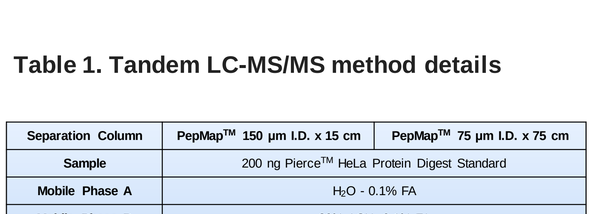
Materials and methods

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. Optimized LC methods use "look-ahead" injections to load sample onto the second column while the separation on the first column is still on-going. They also employ 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

Separation Column	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. Operating 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

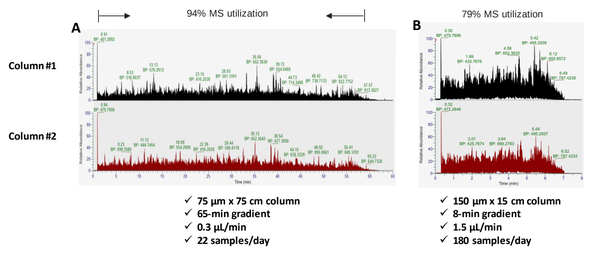


Results

Maximal 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.

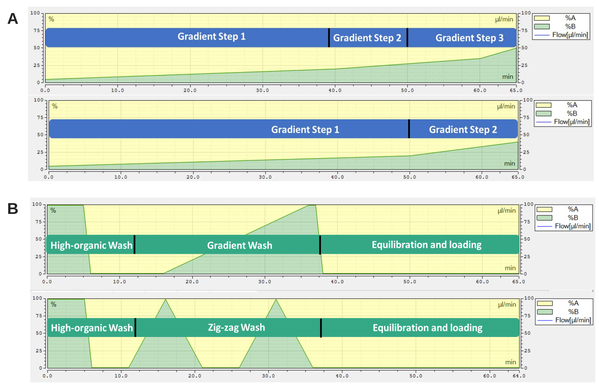
Figure 5. TIC profiles for HeLa peptides separation on column 1 and 2 with 65 min (A) and 8 min (B) methods in tandem direct injection experiments



Method versatility of the tandem direct injection workflow

The tandem workflow permits users to adjust the 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.

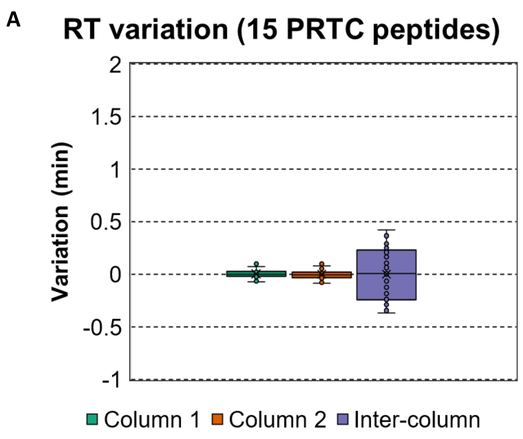
Figure 6. The versatility of defining the gradient for separation (A) and program washing and equilibration cycles (B)

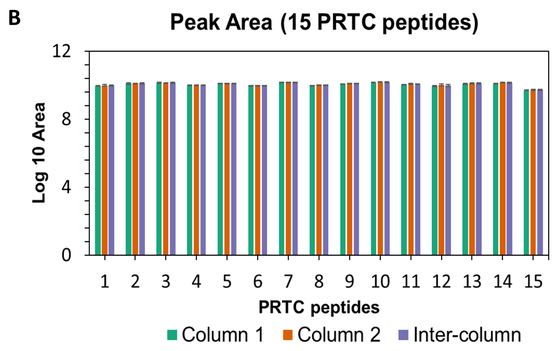


Reproducible intra- and inter-column performance

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. Ecellent 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.

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.

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