Balancing sensitivity and throughput for the analysis of limited sample amounts in proteomics with ultra-low nano-flow LC-MS

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ABSTRACT

Purpose: Systematically investigate the influence of flow-rate and column diameter on the signal intensity and the number of protein and peptide identifications in ultra-low nano-flow LC-MS proteomics. Develop standardized and optimized methods for the high-throughput LC-MS proteomics analysis of limited sample amounts and single cells

Methods: The Thermo Scientific™ Vanquish™ Neo UHPLC system, Thermo Scientific™ PepMap™ Neo columns, and Thermo Scientific™ Orbitrap™ Exploris™ 480 mass spectrometer. Both direct injection and trap-and-elute workflows were developed to showcase maximum sensitivity and throughput for LC-MS analysis using flow rates < 100 nL/min.

Results: Up to 10-fold improvement in sensitivity over nano-LCMS analysis at 300 nL/min enabled identification and quantification of > 2000 protein groups in the single-shot injection of 5 ng of HeLa protein digest and > 200 proteins from 200 pg of HeLa protein digest with single-shot LC-MS of 20 min cycle time (72 samples per 24 hours). Using the trap-and-elute workflow, cycle times as low as 20 min were achieved.

INTRODUCTION

LC-MS profiling of limited sample amounts (e.g. single-cells) requires the highest possible sensitivity. At the same time, the analysis throughput and chromatographic performance must be preserved in order to generate sufficiently high-quality data from large data sets to draw meaningful conclusions. LC-ESI-MS sensitivity is intrinsically linked to both electrospray ionization efficiency and chromatographic scale (Figure 1).

The current approaches to run ultra-low nano-flow LC-MS analysis at flow rates below 100 nL/min with sub 50 µm column IDs require specialized setups, additional hardware, and advanced skills to operate nano-LC systems. Here we demonstrate how next-generation nano-, capillary, and micro-flow UHPLC is designed to run ultra-low nano-flow LCMS analysis using the standard hardware.

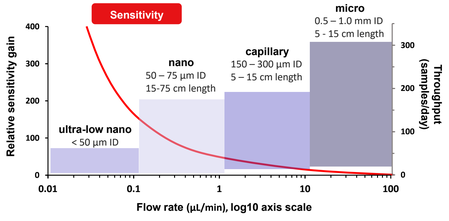


Figure 1. Comparison of relative sensitivity gains of low-flow LC-MS at various nano-, capillary-, and micro-flow rates and corresponding typical throughput.

MATERIALS AND METHODS

Sample Preparation

Thermo Scientific™ Pierce™ HeLa Digest was reconstituted by adding 50 µL of 0.1% formic acid (FA) in water.

Instrumentation

All experiments were performed using a Vanquish Neo UHPLC system interfaced to an Orbitrap Exploris 480 mass spectrometer operated in data-dependent acquisition (DDA) mode. Direct injection methods utilized a Double nanoViper™ PepMap Neo column (20 µm × 150 mm, 2 µm), while trap-and-elute workflows were performed using a Single nanoViper PepMap Neo column (20 µm × 250 mm, 2 µm) with a PepMap trap column (30 µm × 2 mm, 3 µm).

Methods

All experiments were performed using with the following solvents: eluent A – 100% water, 0.1% formic acid; eluent B – 80% acetonitrile/20% water (v/v), 0.1% formic acid. For each method, HeLa digest was injected onto the Vanquish Neo UHPLC system operated in either the direct injection or trap-and-elute workflow in forward flush mode (Figure 2). The sample amount on the column was varied by altering the injection volume.

Data processing and analysis

Acquired data files were processed with Thermo Scientific™ Proteome Discoverer™ Software (version 2.5) using a 2-step Sequest™ HT search algorithm and INFERYS rescoring node. The false discovery rate (FDR) was set below 1% at the peptide and the protein level, respectively.

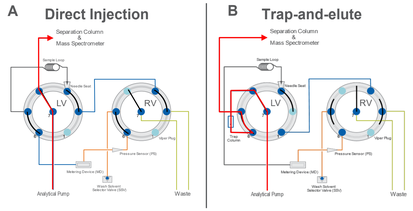
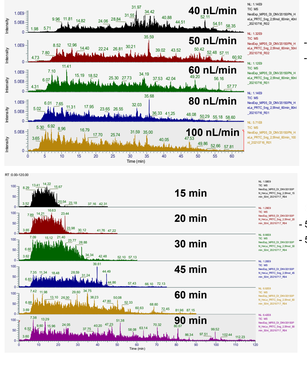


Figure 2. Vanquish Neo UHPLC system configured in the trap-and-elute workflow in backward flush mode.

RESULTS

Optimizing analysis of limited samples

The Vanquish Neo UHPLC system provides the flexibility to optimize ultra-sensitive analysis to maximize the number of protein identifications. To optimize assay performance, flow rate and gradient length were evaluated from 40 - 100 nL/min using gradients with the length from 15 to 90 min, respectively.



A
Flow rate evaluation
- 5 ng HeLa protein digest
- 60-min gradient

B
Gradient length evaluation
- 5 ng HeLa protein digest
- 50 nL/min

Figure 3. Typical TIC profiles for 5 ng of HeLa protein digest on the column. A: flow rates from 40 to 100 nL/min, 60 min gradient length; B: gradient length from 15 to 90 min at flow rate 50 nL/min

DIRECT INJECTION WORKFLOW OPTIMIZATION

The systematic evaluation of results showed that the highest number of peptide and protein identifications for injections of 5 ng of HeLa protein digest is observed with a 90 min gradient (Figure 4). But for the lower loading amount of 1 ng of protein digest, the experiments clearly showed the reduction of identifications for gradients longer than 30 min. This can be caused by increased peak width and reduced peak height, so the sensitivity becomes insufficient for confident peptide identifications. Thus, the lower sample amounts benefit from steeper gradients to boost the MS response and number of identifications.

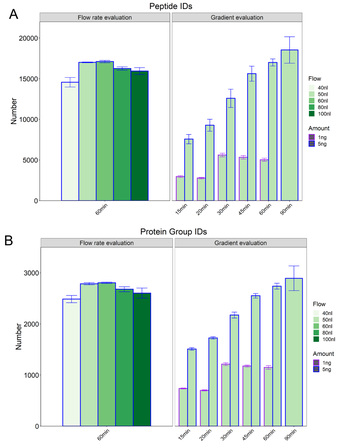


Figure 4. The number of peptides (A) and proteins (B) identified while varying the flow rate from 40 to 100 nL/min with 60 min gradient length or gradient length from 15 to 90 min at flow rate of 50 nL/min

The flow rate change from 40 nL/min to 100 nL/min showed that the maximum number of protein and peptide identifications is achieved with flow rates from 50 to 80 nL/min (Figure 4). While the lower flow rates result in higher ESI response the sensitivity benefits did not translate into practically relevant improvements for single-shot DDA analysis due to elution delay and peak broadening.

TRAP-AND-ELUTE WORKFLOW OPTIMIZATION

Due to long injection times for the direct injection workflow, e.g. the complete loading of 1.0 µL sample with Loading Volume set to Automatic takes approximately 30 min when sample loading cannot be accelerated due to column pressure limitation. We optimized high-throughput trap-and-elute standardized workflows for fast profiling of limited sample amounts and compared results obtained with 50 nL/min and 80 nL/min flow rates (Figure 5). Interestingly, the better separation profiles and sharper peaks translated into more proteins and peptides identified at 80 nL/min.

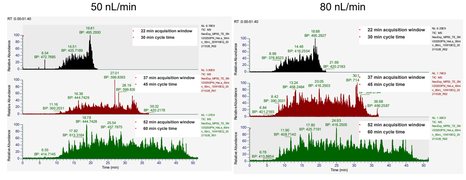


Figure 5. The typical TIC profiles for separation of 1 ng of HeLa protein digest with trap-and-elute workflow using 50 nL/min and 80 nL/min flow rate

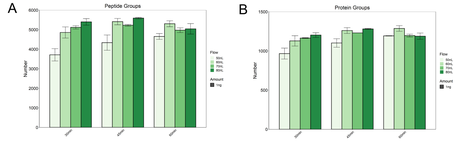


Figure 6. The effect of flow-rate and gradient length on the number of peptide (A) and protein (B) identifications for single-shot LC-MS analysis of 1 ng HeLa protein digest

With the focus on high throughput analysis of limited sample amounts, we created a set of ultra-low nano-flow LC-MS methods for the single-shot analysis (Figure 7). With the overhead time of around 10 min, standardized methods allow running from 72 to 24 samples per day. The achieved throughput in combination with multiplexing, e.g. TMT labeling, can be used to analyze ca. 1000 single cells per 24 hours.

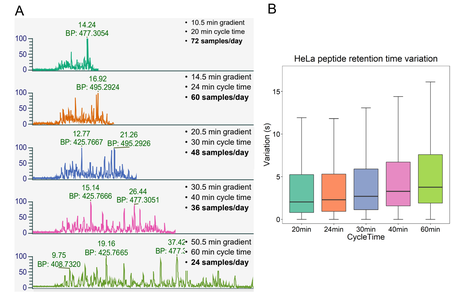


Figure 7. Chromatograms demonstrating method throughput and high MS utilization using the optimized trap-and-elute workflows with 80 nL/min flow rate

CONCLUSIONS

We evaluated the suitability of the Vanquish Neo UHPLC system for the analysis of limited sample amounts using ultra-low nano-flow rates and columns with an internal diameter of 20 µm and trap columns with internal diameter of 30 µm.

- The default Vanquish Neo UHPLC system configuration for nanoLC separations can be successfully used for ultra-low nano-flow LC-MS analysis of limited sample amounts
- The optimal flow rates for the analysis of limited sample amounts are in the range of 50-80 nL/min for direct and trap-and-elute injections
- Trap-and-elute injection workflow under optimal separation and sample injection conditions allows performing analysis of 24 to 72 samples/day with high MS utilization

TRADEMARKS/LICENSING

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