Beyond the Boundaries of LC-MS Sensitivity and Throughput with the Next Generation All-In-One Nano-, Capillary-, and Micro-Flow UHPLC System

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ABSTRACT

Purpose: Comprehensively evaluate the Thermo Scientific™ Vanquish™ Neo UHPLC system for bottom-up proteomics.

Methods: The Vanquish Neo UHPLC system, Thermo Scientific™ EASY-Spray™ PepMap™ Neo or Thermo Scientific™ Double nanoViper™ PepMap™ Neo columns, and Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer were used for bottom-up proteomics analysis. Targeted quantification was performed using a Thermo Scientific™ Acclaim™ PepMap™ column and Thermo Scientific™ TSQ Altis™ mass spectrometer.

Results: We identified > 7k proteins and 90k peptides in data-dependent acquisition experiment. High-throughput LC-MS methods using nano and capillary columns enabled analysis of up to 180 samples per day. Between Vanquish Neo instruments and sites ca. 80% of peptides were reproducibly identified and quantified. Lastly, micro-flow capabilities enabled ultra-robust analysis of large sample cohorts.

INTRODUCTION

The limitations of existing low-flow LC technologies are related to insufficient hardware robustness, complex operation, limited versatility, and the skill required to run proteomics experiments. The variety of modern LC-MS applications from the analysis of limited sample amounts to high-throughput proteome profiling required the development of a next-generation UHPLC system. We present here the all-in-one UHPLC system designed for high-sensitivity nano-, capillary- and micro-flow LC-MS applications. Vanquish Neo UHPLC system (Figure 1) features include:

- Thermo Scientific™ ProFlow™ XR technology active flow control across the entire flow range for enhanced gradient accuracy and precision.
- SmartInject technology for high retention time precision and extending column lifetimes.
- Fast sample loading and column equilibration for reducing analysis time and increasing mass spectrometer utilization.
- Vial-bottom detection technology for injection from limited sample volumes with limited sample waste.
- A high-precision metering device for injection volumes from 10 nL to 100 µL and multi-draw for extended volumes.
- Multi-wash injection routines for reduced system carryover
- The Vanquish User Interface for smart system operation, status monitoring, and automatic procedures.
- Smart guided method creation with integrated consumable and fluidic parameters.

Figure 1. Vanquish Neo UHPLC system, Orbitrap Exploris 480 MS and examples of EASY-Spray and Double nanoViper PepMap Neo columns used in this study

MATERIALS AND METHODS

All experiments were performed using a Vanquish Neo UHPLC system 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 more detailed information, please refer to references 1-4.

Nano- and capillary-flow LC-MS

NanoLC-MS bottom-up proteomics experiments were performed using Vanquish Neo system interfaced with an Orbitrap Exploris 480 mass spectrometer operated in DDA mode. EASY-spray PepMap Neo columns (75 µm × 500 mm and 75 µm × 750 mm, 2 µm) were used in the direct injection workflow. Samples consisted of Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard (A47996) at a concentration of 400 ng/µL HeLa with 200 fmol/µL PRTC.

Nano- and capillary-flow LC-MS methods for high-throughput proteome profiling experiments were performed using Vanquish Neo system interfaced with an Orbitrap Exploris 480 mass spectrometer operated in DDA mode. An EASY-spray PepMap Neo column (75 µm × 150 mm, 2 µm) was used in the trap-and-elute workflow. Samples consisted of HeLa Digest/PRTC Standard (A47996) at a concentration of 200 ng/µL HeLa with 100 fmol/µL PRTC.

Robust, long-term nano-flow LC separations experiments were performed using a Vanquish Neo system including a thermostatted column compartment and VWD detector. A Double nanoViper PepMap Neo column (75 µm × 500 mm, 2 µm dp) was used in the direct injection workflow. Samples consisted of 1 pmol/µL BSA protein digest.

Micro-flow LC-MS

Targeted, high-throughput peptide quantification experiments were performed using a Vanquish Neo system including a thermostatted column compartment interfaced to a TSQ Altis triple quadrupole mass spectrometer. An Acclaim PepMap 1.0 mm × 15 cm column was used in the direct injection workflow. Samples consisted of HeLa Digest/PRTC Standard (A47996) at a concentration of 50 ng/µL HeLa and 25 fmol/µL PRTC.

Figure 2. Fast sample loading and equilibration improves throughput on 75 µm I.D. × 50 cm and 75 µm I.D. × 75 cm columns.

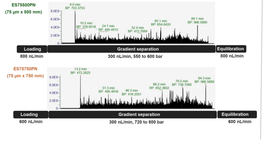
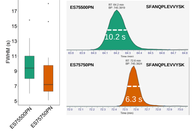


Figure 3. Comparison of FWHM for 75 cm vs. 50 cm columns in a 90-min gradient with 100 fmol PRTC spiked in HeLa protein digest reveals reductions of up to 2 s for 50% of peptides (A) and up to 4 s for certain peptides (B).



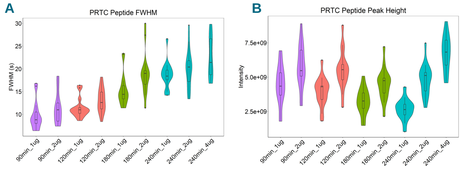
RESULTS

Setting new performance standards in bottom-up proteomics[1]

While 75 µm I.D. × 50 cm columns have traditionally been associated with discover proteomics, 75 µm I.D. × 75 cm columns provide an opportunity for deeper proteome profiling due the potential for higher peak capacities. Widespread adoption of such columns has been hampered by the maximum pressure ratings of available nanoLC system, limiting method throughput. The Vanquish Neo UHPLC system and separation columns, capable of robust operation at 1500 bar, can significantly accelerate the sample loading and column equilibration steps for both 50 cm and 75 cm long nano-columns (Figure 2). The 75 cm column outperformed the 50 cm in a 90-min gradient by reducing the FWHM by 2 seconds for 50% of the peptide peaks (Figure 3).

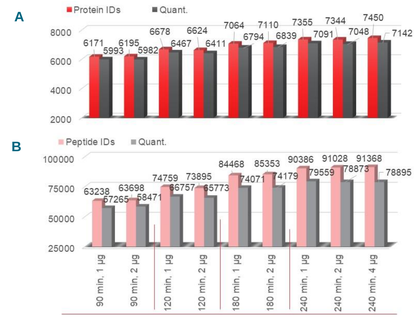
Single shot nanoLC-MS analyses requires the highest possible separation efficiency to maximize the number of identified peptides and proteins. Because peak capacity is proportional to gradient time, increasing gradient length also provides increased peak capacity. Despite the almost linear increase of FWHM with gradient length (Figure 4), the number of peptide and protein identifications tend to increase due to the extra time available for precursor ion isolation and fragmentation. While sensitivity loss due to peak broadening in longer gradients can be compensated for with larger sample amounts, the effect of column overloading on FWHM limits such gains (Figure 4).

Figure 4. Dependency of FWHM (A) and peak height (B) on gradient length using a constant flow rate (250 nL/min) and different loading amounts.



By employing a 240-min gradient and loading 1 µg HeLa protein digest, more than 7,100 proteins (1% FDR) with ca. 80K peptides were successfully quantified after separation on a 75 cm nano Column (Figure 5). Interestingly, no significant improvement in peptide or protein IDs was observed with increased loading amount. These data illustrate the power of high-resolution chromatography coupled with HRAM MS in boosting proteome depth and coverage.

Figure 5. The number of proteins (A) and peptides (B) identified and quantified by varying gradient length from 90 to 240 min and loading amount from 1 to 4 µg.



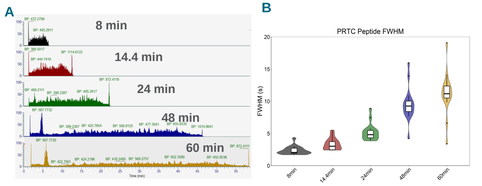
*75 µm x 75 cm, 2 µm, 3 replicates; including match between runs; 2-step Sequest™ HT and INFERYS, < 1% FDR

Nano- and capillary-flow LC-MS methods for high-throughput proteome profiling[2]

To determine the utility of the Vanquish Neo UHPLC system for high-throughput, bottom-up proteome profiling a total of 5 methods with cycle times from ranging from 8 to 60 min were developed and evaluated using PRTC peptides spiked into a HeLa protein digest (Figure 6). MS utilization ranged from 68% to 95% with throughput of up to 180 samples/day.

All methods yielded excellent separation profiles (Figure 6A). FWHM obtained for the PRTC peptides reached < 3 s for the 8 min method and gradually increased with increasing gradient duration and the corresponding reduction in flow rate (Figure 6B).

Figure 6. Chromatograms generated from PRTC peptides spiked into 200 ng HeLa digest for 5 high-throughput methods (A). Median FWHM for 15 PRTC peptides ranged from 2 to 11 s (B).



Robust, long-term nano-flow LC separations[3]

Long-term robustness was evaluated with continuous analyses of 1 pmol injections of bovine serum albumin (BSA) protein digest on a 75 µm I.D. × 50 cm PepMap Neo column using a 90-minute nano-flow separation gradient and UV detection (Figure 7 and 8).

Figure 7. Representative LC-UV chromatogram for a 1 pmol injection of BSA protein digest onto a 75 µm × 50 cm PepMap Neo column. The 8 peaks selected for evaluation are highlighted.

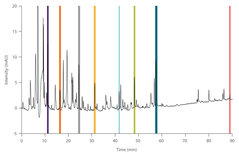
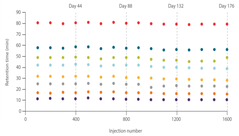


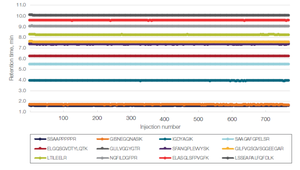
Figure 8. The retention time for 8 selected peptides from 1,600 injections of BSA protein digest over 176 days (~6 months). Each marker represents a mean of 100 injections.



Micro-flow LC-MS/MS for targeted, high-throughput peptide quantification[4]

Improvements in modern mass spectrometer scan speed and sensitivity have paved the way for high-throughput proteome screening. However, large sample cohort analyses also require high micro-flow LC injection and retention time reproducibility which, until now, have been limiting factors. Here, a 14.4 min quantitative micro-flow LC-MS method was developed and evaluated for run-to-run reproducibility across 760 repeated injections over 7.5 days. High retention time stability was observed for 12 PRTC peptides with the RSD of 11 peptides < 0.15% and one at 0.31% (Figure 9). Peak area reproducibility was also assessed. The sample was exchanged after 452 injections due to sample depletion. As a result, peak area stability was considered separately for the first 452 and last 308 injections

Figure 9. Retention time stability of 12 PRTC peptides for all sample injections of the sequence.



CONCLUSIONS

We comprehensively evaluated the Vanquish Neo UHPLC system's sample handling and sample separation technologies for bottom-up proteomics experiments. Novel design of LC hardware extends the boundaries of high-sensitivity LC-MS analysis and enables applications from discovery to validation without compromising robustness, ease-of-use and quality of results.

- The Vanquish Neo sets new performance records in single-shot, bottom-up proteomics with over 7,000 protein and 80k peptide identifications.
- High throughput methods were developed on 15 cm nano and capillary columns with throughputs from 24 to 180 samples/day, demonstrating the versatility of the Vanquish Neo for optimizing performance and throughput.
- Long-term nano-flow LC robustness was evaluated with 1600 injections over 6 months, demonstrating high retention time reproducibility.
- Micro-flow LC-MS was performed for high-throughput, large cohort bottom-up sample analysis demonstrating high peak area precision for 12 PRTC peptides.

REFERENCES

1. Zheng, R. et al. Vanquish Neo UHPLC system sets new performance standards for single-shot nanoLCMS bottom-up proteomics. TN74152 (2021)
2. Zheng, R. et al. Fast, sensitive, and reproducible nano- and capillary-flow LCMS methods for high−throughput proteome profiling using the Vanquish Neo UHPLC system hyphenated with the Orbitrap Exploris 480 MS. TN000138 (2021)
3. Pynn, C. et al. Robust long-term Vanquish Neo UHPLC system operation enabling high-performance high- pressure nanoLC separations. TN000172 (2021)
4. Meding, S. et al. Ultra-robust micro-flow LC-MS/MS for targeted high-throughput peptide quantification using the Vanquish Neo UHPLC system. TN74161 (2021)

TRADEMARKS/LICENSING

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