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Proteomics

Multi-draw: Enabling large volume injections for lyophilization-free LC-MS proteomics workflows on the Vanquish Neo UHPLC system

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Keywords

Vanquish Neo UHPLC system, Orbitrap Exploris 480 mass spectrometer, LC-MS, EASY-Spray PepMap Neo column, EASY-Spray PepMap Neo trap column, multi-draw, large volume injection, trap-and-elute, bottom-up proteomics, HeLa protein digest, PRTC standard, capillary-flow LC, lyophilization-free, high-throughput

Goal

Demonstrate the performance of the multi-draw functionality using the Thermo Scientific™ Vanquish™ Neo UHPLC system for large volume injections in bottom-up proteomics experiments within the trap-and-elute workflow.

Introduction

Most LC-MS-based bottom-up proteomics workflows utilize labor-intensive sample preparation protocols, including protein digestion and sample cleanup followed by lyophilization. Dried samples are then resuspended in a smaller volume of a buffer to ensure injection solvent compatibility with reversed-phase chromatography and to boost method sensitivity (i.e., identifications) and throughput by injecting greater peptide mass in lower volume on the separation or trap column. While currently the most popular approach, lyophilization and reconstitution cycles increase workload and introduce the possibility of loss of peptides with limited aqueous solubility prior to LC-MS analysis.

Peptide loss can be potentially avoided by skipping the conventional lyophilization and reconstitution cycle. Instead, samples are simply diluted in an LC-friendly solvent and injected onto a trap column for fast loading. However, one major concern when injecting large volumes is the breakthrough of hydrophilic peptides during trapping. Breakthrough can result in loss of hydrophilic peptides, poor peak shapes, and ultimately issues with reproducibility. An appropriate injection buffer could mitigate peptide breakthrough by ensuring adequate retention during the sample loading phase.

In this work, we demonstrate a new workflow using a Vanquish Neo UHPLC system for high-speed and high-efficiency loading of large volume samples onto a 300 µm i.d. x 0.5 cm trap column. The multi-draw functionality, in combination with a 100 µL sample loop, extends the injection volume capacity of the system to 500 µL, circumventing

the need for sample lyophilization and reconstitution. Trifluoroacetic acid (TFA, 0.1% (v/v)) was evaluated as a strong ion-pairing reagent in the loading buffer and sample solvent to prevent peptide breakthrough. Here we demonstrate how multi-draw and TFA ion-pairing were successfully applied to a previously published capillary-flow LC-MS method for bottom-up proteomics in the trap-and-elute workflow.[1] The large volume injection setup exhibited equivalent chromatographic performance and peptide/protein identifications compared to the typical (5 µL) injection volumes employed in trap-and-elute workflows.

Materials and methods

Sample preparation

Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard was reconstituted by adding 50 µL of 0.1% formic acid (FA) in water to get 200 ng/µL HeLa digest with 100 fmol/µL PRTC peptides. The vial was subsequently sonicated for 2 min, followed by 10 sample aspiration and release cycles with a pipette to dissolve the standard completely. Samples were diluted in water (0.1% TFA, v/v) to 5 ng/µL HeLa digest with 2.5 fmol/µL PRTC.

Data acquisition and processing

LC-MS analyses were performed using a Vanquish Neo UHPLC system interfaced to a Thermo Scientific™ Orbitrap Exploris™ 480 mass spectrometer operated in data-dependent acquisition (DDA) mode.[1] The system was configured for the trap-and-elute nano/cap workflow using a Thermo Scientific™ EASY-Spray PepMap™ Neo column (75 µm i.d. x 150 mm, 2 µm) and Thermo Scientific™ PepMap™ Neo Trap Cartridge (300 µm i.d. x 5 mm, 5 µm). Methods are available for download from the Thermo Scientific™ AppsLab library. Data was acquired using Thermo Scientific™ Standard Instrument Integration (SII) 1.7 for Xcalibur™ software, followed by .raw file processing with Thermo Scientific™ Proteome Discoverer™ 2.5 software using a 2-step SEQUEST™ HT search algorithm and INFERYS rescoring node. The false discovery rate (FDR) was set below 1% at both the peptide and the protein levels. Further data analysis and plotting were performed with R script.[2]

Table 2. Gradient for 180 samples/day method[1]

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	1.3	4
3.7	3.7		20
5.5	1.8		35
Column wash phase			
5.9	0.4	1.3	99
6.6	0.7		99

Table 3. Vanquish Neo and Orbitrap Exploris LC-MS system

Description	Part Number
Vanquish Neo UHPLC system (Binary Pump N, Split Sampler NT, Solvent Rack, System Base with drawer, Ship kit)	VN-S10-A-01
Vanquish System Controller (required)	6036.1130
Vanquish Display (required)	6036.1180
Orbitrap Exploris 480 mass spectrometer	BRE725539
Thermo Scientific™ EASY-Spray™ source	ES082
EASY-Spray PepMap Neo column (75 µm i.d. x 150 mm, 2 µm)	ES75150PN
PepMap Neo trap cartridge (300 µm i.d. x 5 mm, 5 µm)	174500
PepMap Neo trap cartridge holder with nanoViper capillaries	174502
Sample loop, 100 µL	6252.1950
Total recovery vial 1.5 mL	6PSV9-TR1
Talcum-free cap screw 9 mm	6PSC9STB1

Table 1. Solvents and additives

Reagent	Grade	Supplier	Part number
HeLa Digest/PRTC standard (10 µg/5 pmol)	N/A	Thermo Scientific™ Pierce™	A47996
80% Acetonitrile with 0.1% FA	Optima™ LC-MS	Fisher Chemical™	LS122500
Isopropanol	Optima™ LC-MS	Fisher Chemical™	A461-212
Formic acid	Optima™ LC-MS	Fisher Chemical™	A117-50
Water with 0.1% formic acid	Optima™ LC-MS	Fisher Chemical™	LS118500
Trifluoroacetic acid	LC-MS	Thermo Scientific™ Pierce™	85183
Water	Optima™ LC-MS	Fisher Chemical™	W6212

Table 4. LC-MS method solvents

Solvent	Composition
Eluent A	100% water, 0.1% formic acid
Eluent B	80% acetonitrile, 20% water (v/v), 0.1% formic acid
Weak metering device wash liquid	100% water, 0.1% trifluoroacetic acid
Strong metering device wash liquid	80% acetonitrile, 20% water (v/v), 0.1% formic acid
Weak (outer) needle wash liquid	100% water, 0.1% formic acid
Strong (outer) needle wash liquid	80% acetonitrile, 20% water (v/v), 0.1% formic acid

Table 5. LC parameters for large volume injection

Parameter	Value
Sample loading	
Mode	CombinedControl
Flow	200 µL/min
Pressure	800 bar
Loading volume*	Automatic (5 µL)
Sample pick–up	
Outer needle wash mode*	After draw
Outer needle wash time (strong)*	3.0 s
Outer needle wash speed (strong)*	80.0 µL/s
Outer needle wash time (weak)*	5.0 s
Outer needle wash speed (weak)*	80.0 µL/s
Draw speed	20 µL/s
Draw delay*	2.0 s
Dispense speed*	5.0 µL/s
Vial bottom detection*	Enabled
Column equilibration	
Fast equilibration	Disabled
Mode	–
Pressure	–
Equilibration factor	0
Trap column	
Fast wash and equilibration*	Enabled
Wash factor	50
Equilibration factor*	Automatic (i.e., 2)
Mode	CombinedControl
Flow	200 µL/min
Pressure	800 bar
Trap flush direction	Backward
Temperature	
Thermo Scientific™ EASY-Spray™ column temperature	50 °C
Autosampler temperature*	7 °C
Trap cartridge*	Room temperature (~23 °C)
Sampler advanced settings	
Wash cycle time	Fast

*System default values

Results and discussion

Working principle of multi-draw function

Vanquish Neo UHPLC system multi-draw functionality supports large volume injections for trap-and-elute workflows through iterative sample pick-up. After each aspiration, the respective sample volume is transferred to the trap column. The process is repeated until the full sample volume has been loaded onto the trap column. TFA (0.1%, v/v) was used in place of formic acid in the loading solvent (weak wash liquid) and the sample diluent to provide increased peptide retention on the trap column through strong ion-pairing interactions.

A 100 µL loop must be installed (VSC UI script A07: Change Sample Loop) to enable the multi-draw functionality. All the other parts of the injection cycle (e.g., sample aspiration and loading) are executed automatically in Xcalibur software without user interaction once an injection volume >100 µL is entered into the sequence table.

To evaluate multi-draw for large volume injections, 1 µg of HeLa digest was loaded onto the trap column at two different concentrations, thus requiring two different aspiration volumes:

1. Standard volume injection: 5 µL at 200 ng/µL

2. Large volume injection: 500 µL at 2 ng/µL

Injections of 5 µL (200 ng/µL) were performed using a standard single injection program,[1] while 500 µL (2 ng/µL) injections utilized several multi-draw cycles to aspirate and load sample onto the trap column automatically, requiring 2 min/cycle (additional 14 min of cycle time, Figure 1A). It should be noted that the maximum injection volume of 500 µL was chosen explicitly to demonstrate the efficacy of the Vanquish Neo system multi-draw functionality. The aspiration of lower injection volumes results in decreased cycle time and increased sample throughput.

Multi-draw supports deep coverage proteome profiling

Both 5 µL and 500 µL injections yielded similar signal intensity and chromatographic profiles (Figure 1A). Additionally, retention times of 15 PRTC peptides extracted from the chromatograms were consistent across injection volumes (Figure 1B).

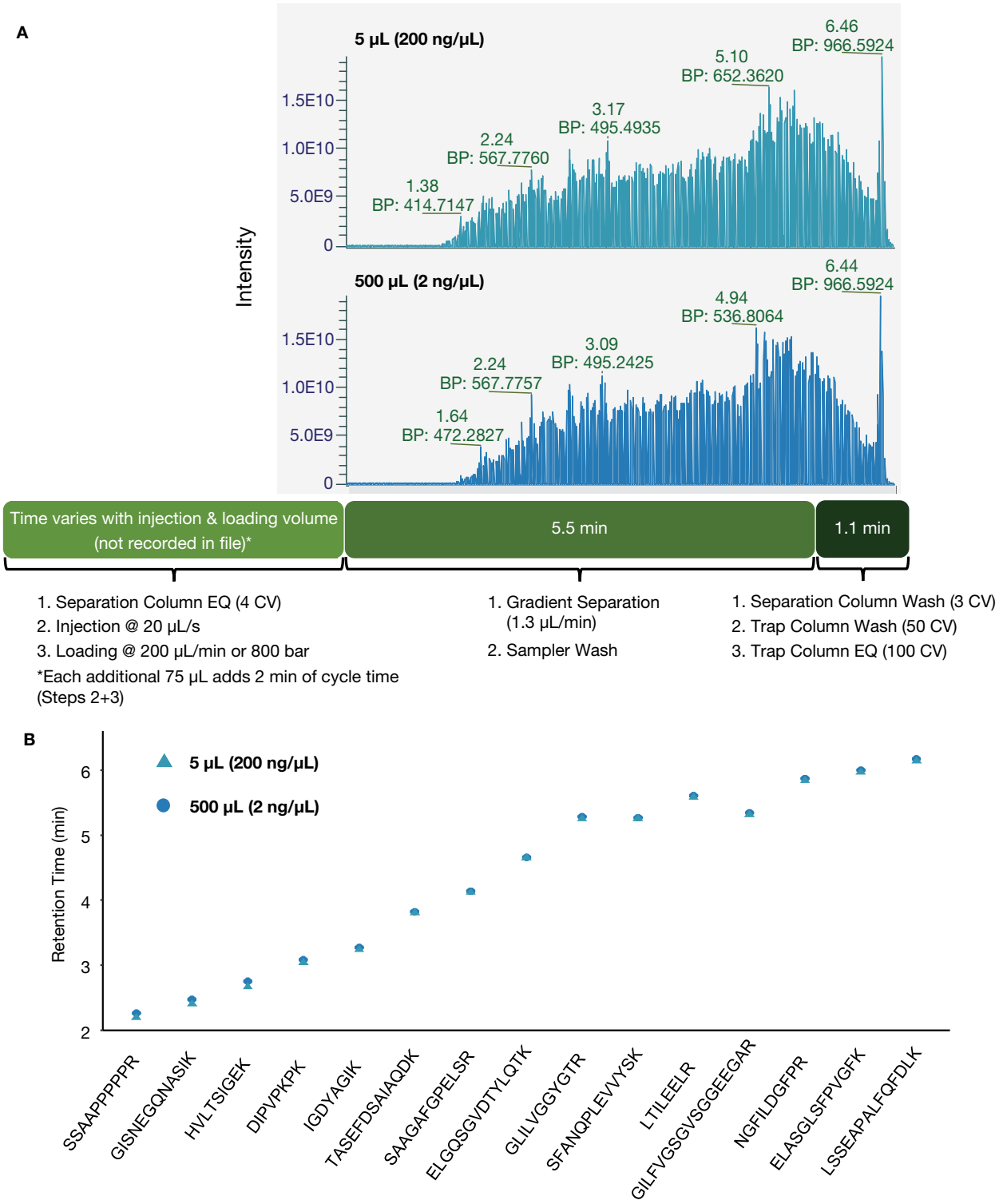

Figure 1. Evaluating multi-draw for large volume injections. (A) Total ion chromatograms (TICs) of 1 µg HeLa protein digest obtained after injection of 5 µL of 200 ng/µL (top) or 500 µL of 2 ng/µL (bottom) onto the same trap cartridge, including a breakdown of the full method cycle time. (B) Retention times of 15 PRTC peptides extracted from each separation.

In addition to chromatographic performance, the depth of proteome coverage was evaluated for multi-draw large volume injections. Figure 2 shows agreement between the number of peptides and proteins identified for 5 and 500 µL injection volumes, indicating that an equivalent total quantity of sample was loaded onto the trap column (and ultimately analytical column) for each method.

Lastly, quantitative performance was compared between injection volumes. The high correlation of peptide peak areas between 5 µL (200 ng/µL, Figure 3A-D) and 500 µL (2 ng/µL, Figure 3E-I) injections illustrates no systematic bias due to peptide breakthrough when using multi-draw with 0.1% TFA as the loading buffer. Further examination of protein abundance provides evidence of no systematic bias during multi-draw large volume injections (Figure 4).

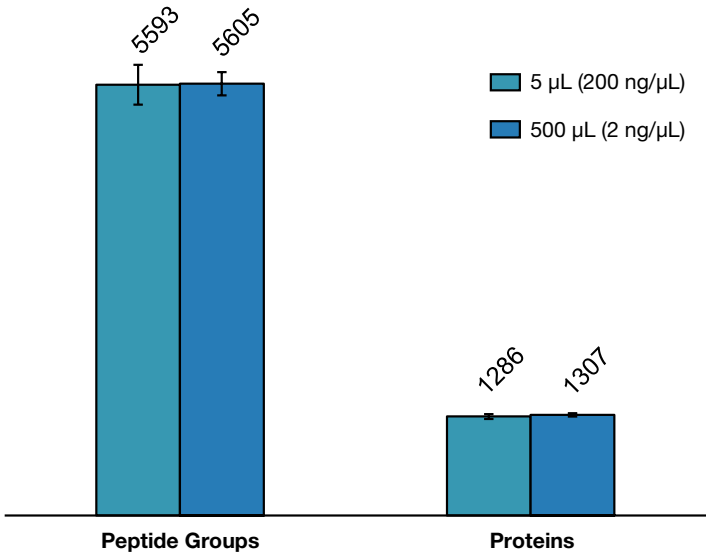

Figure 2. The number of peptide groups and proteins identified for standard and multi-draw injections (N = 5). FDR of 1% was used for protein identification.

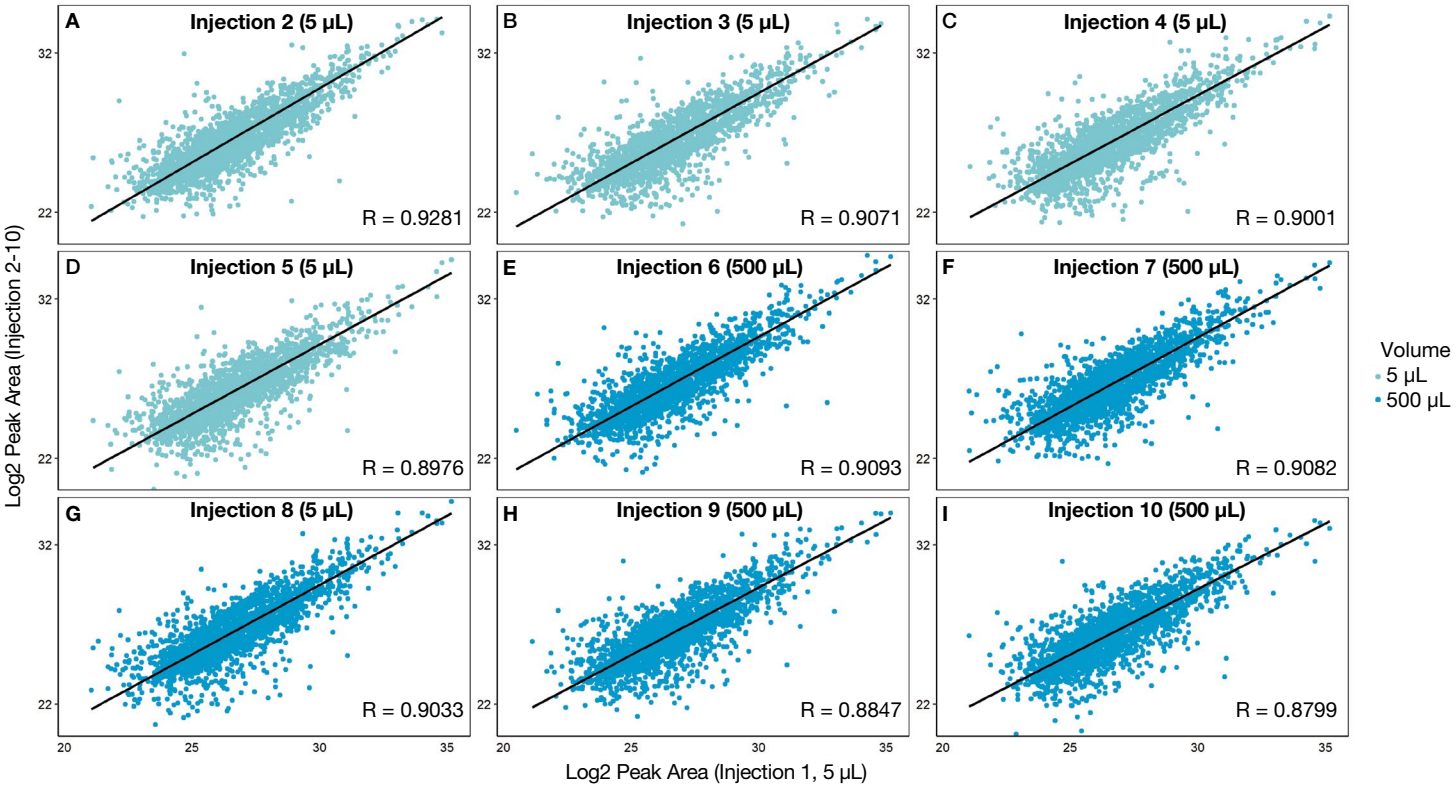

Figure 3. The correlation between peptide peak areas for injection replicates of HeLa protein digest. Green plots show the correlation of four subsequent 5 µL injection replicates with the first 5 µL injection (A-D), while blue plots show the correlation of each of the five 500 µL injection replicates with the initial 5 µL injection (E-I).

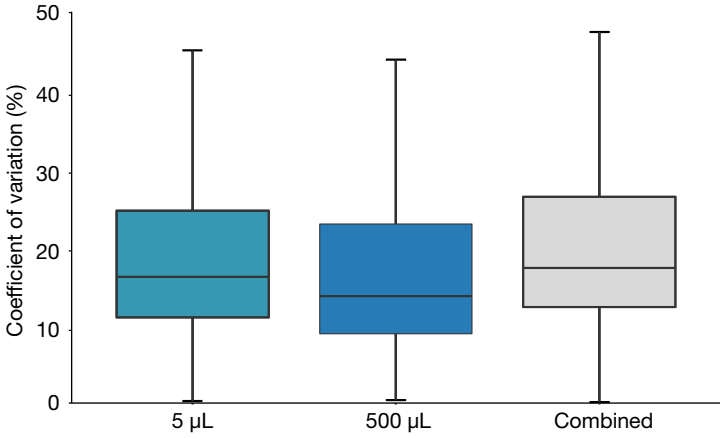

Figure 4. Protein abundance variation for 5 µL and 500 µL replicate injections (N = 5). Gray represents the combined abundance variation for all 5 µL and 500 µL injections.

Conclusions

The multi-draw functionality of the Vanquish Neo UHPLC system provides accurate and precise high-volume sample aspiration, enabling the user to perform injections from 10 nL to 500 µL with the following attributes:

- Automatic multi-draw execution when employing a 100 µL sample loop without user programming effort
- High speed sample injection and loading (sample aspiration at 20 µL/s and loading with CombinedControl at 200 µL/min and 800 bar) when using a 300 µm i.d. x 0.5 cm trap cartridge
- Retention of hydrophilic peptides when combined with 0.1% TFA for sample reconstitution and loading
- Equivalent peptide and protein identification and quantification for diluted HeLa digest (2 ng/µL) loaded in a large injection volume (500 µL) and concentrated HeLa digest (200 ng/µL) loaded with a small injection volume (5 µL)

Overall, the Vanquish Neo multi-draw functionality enables a dilute-and-inject workflow (<2% final ACN concentration in sample) for bottom-up proteomics analysis that precludes the need for lyophilization and reconstitution during sample preparation prior to LC-MS analysis.

References

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