LC-MS

TECHNICAL NOTE

Robust long-term Vanquish Neo UHPLC system operation enabling high-performance high-pressure nanoLC separations

Authors

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Keywords

Thermo Scientific Vanquish Neo UHPLC system, Thermo Scientific PepMap Neo columns, nanoLC, bottom-up proteomics, robustness, 1500 bar

Goal

Demonstrate the long-term robustness and consistent chromatographic performance of the next-generation Thermo Scientific™ Vanquish™ Neo UHPLC System under nanoLC conditions for bottom-up proteome profiling using a 75 µm I.D. × 50 cm Thermo Scientific™ PepMap™ Neo Column.

Introduction

Nano-flow LC-MS analysis employing long columns and gradients at nano-flow rates has long been established as the method of choice for bottom-up, discovery proteomics. Nevertheless, the challenges associated with nano-flow LC have hitherto limited its application to the field of academic research. The main concerns associated with the technique include locating and eliminating the sources of dead volume, leaks, and capillary or column blockages, combined with the struggle to generate reproducible data. Together, such obstacles can result in the loss of precious time and irreplaceable samples. Another caveat of nanoLC-MS applications is their limited sample throughput and MS utilization. Lower flow rates result in proportionally long periods of MS idle time due to time-consuming sample loading and column equilibration particularly when long separation columns are employed. The latest low-flow UHPLC systems and columns have the potential to overcome many of these challenges and to usher in a new era of robustness and standardization in the field of low-flow LC-MS based analytics.

In this Technical Note, we tested the Vanquish Neo UHPLC system for robust and consistent long-term LC separation performance under conditions typically adopted for deep dive proteomics experiments. We carried out continuous analyses of bovine serum albumin (BSA) protein digest on a 75 µm I.D. × 50 cm long PepMap Neo column using a 90-minute nano-flow separation gradient. To reduce the productivity limits described above, the system was operated in fast column loading and equilibration mode exploiting the full 1500 bar back pressure capability of the system and in turn reducing analysis cycle time.

A total of 1600 injection cycles of BSA protein digest were performed over 176 days of continuous operation using a single Thermo Scientific™ PepMap™ Neo Column. The analyses were paused only to replace solvents, add fresh sample vials (once per 100 injections), and run system diagnostics to confirm full system functionality.

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Experimental materials and methods

Sample preparation

CAM-modified trypsin-digested BSA MS Standard (500 pmol/vial, New England Biolabs, PN P8108S) was reconstituted by adding 500 µL of 0.1% formic acid (FA) in water. The vial was subsequently sonicated for 2 min, followed by mixing with a pipette to fully reconstitute the sample. The final sample concentration was 1 pmol/µL BSA protein digest.

Consumables

- Fisher Scientific™ LC-MS grade water with 0.1% formic acid (P/N LS118-500)
- Fisher Scientific™ LC-MS grade 80% acetonitrile with 0.1% formic acid (P/N LS122-500)
- Fisher Scientific™ LC-MS grade formic acid (FA) (P/N A117-50)
- Fisher Scientific™ LC-MS grade isopropanol (P/N A461-212)

Table 1. The Vanquish Neo UHPLC system including thermostatted column compartment and UV detector, fluidics, and accessories

Description	#	Part number
Vanquish Neo UHPLC system Binary pump N, Split sampler NT, solvent rack, Vanquish system controller, system base with drawer, ship kit	1	VN-S10-A-01
Vanquish display (required)	1	6036.1180
Column compartment N	1	VN-C10-A-01
Vanquish VWD C detector	1	VC-D40-A-01
3 nL flow cell	1	6074.0270
Vial and septa kit, 100/pack of • Vial 0.2 ml amber TPX screw 9 mm short thread conical glass insert • Cap screw 9 mm black PP white silicone/red PTFE septa bonded 1.0 mm	1	6PK1655
Thermo Scientific™ Double nanoViper™ PepMap™ Neo Column 75 µm × 500 mm, 2 µm, 1500 bar column	1	DNV75500PN
MicroTight® Adapter for connecting 1/16" to 1/32" fittings	1	00109-02-00055
Sleeves for connecting 280 µm O.D. capillary to MicroTight Adapter union, 10 pcs	1	SC903

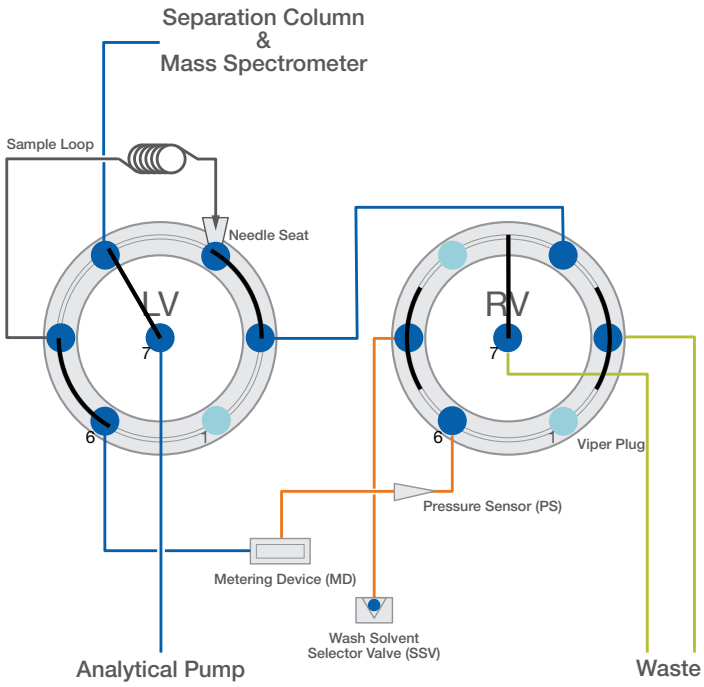

Figure 1. Valve and fluidic configuration for the Vanquish Neo UHPLC system direct injection workflow

The separation column was placed in the thermostatted column compartment. The left autosampler valve was connected to the inlet of the column via a 20 µm × 55 cm nanoViper capillary (PN 6250.5260) and viper union (PN 6040.2304), both of which are supplied in the system ship kit. The nanoViper outlet of the separation column was connected to the nano UV cell inlet using the MicroTight adaptor union and sleeve described in Table 1.

LC solvents and system temperature settings

The solvents and temperature parameters are given in Table 2.

Table 2. Solvents and additives

Solvent	Composition
Mobile phase A	H_2O with 0.1% FA
Mobile phase B	80/20 (%, v/v) ACN/H_2O with 0.1% FA
Weak wash liquid (metering device)	H_2O with 0.1% FA
Strong wash liquid (metering device)	80/20 (%, v/v) ACN/H_2O with 0.1% FA
Weak wash liquid (wash port)	H_2O with 0.1% FA
Strong wash liquid (wash port)	80/20 (%, v/v) ACN/H_2O with 0.1% FA
Rear seal wash buffer	25/75 (%, v/v) H_2O/Isopropanol with 0.1% FA

FA= Formic acid, ACN = Acetonitrile

Vanquish Neo UHPLC system method parameters

The generic parameters for sample aspiration, loading, and column equilibration are shown in Table 3. The LC gradient separation parameters are shown in Table 4.

Table 3. Vanquish Neo UHPLC system parameters

	Parameter	Value
Sample Loading	Fast loading	Enabled
	Mode	Pressure Control
	Pressure	1500 bar
	Loading volume*	Automatic
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	0.2 µL/s
	Draw delay	2.0 s
	Dispense speed	5.0 µL/s
	Vial bottom detection	Enabled
Column Equilibration	Fast equilibration	Enabled
	Mode	Pressure Control
	Pressure	1500 bar
	Equilibration factor	2.0
Temperature	Column compartment	50 °C
	Autosampler	7 °C

*System default values

Table 4. Pump gradient settings

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.3	1.0
0.1	0.1	0.3	6.0
60.1	60	0.3	20.0
90.1	30	0.3	35
Column wash phase			
91.1	1.0	0.3	99
100	8.9	0.3	99

UV data acquisition parameters

UV absorption data were recorded using a wavelength of 214 nm and a data collection rate of 4 Hz.

Data acquisition and processing

LC-UV data were acquired and analyzed using the Thermo Scientific™ Chromeleon™ Chromatography Data System (version 7.2.10 MUd). Peak properties (full width at half maximum—FWHM, retention time—RT) were extracted for 8 selected BSA peptides (Figure 2) using the Cobra peak detection algorithm.

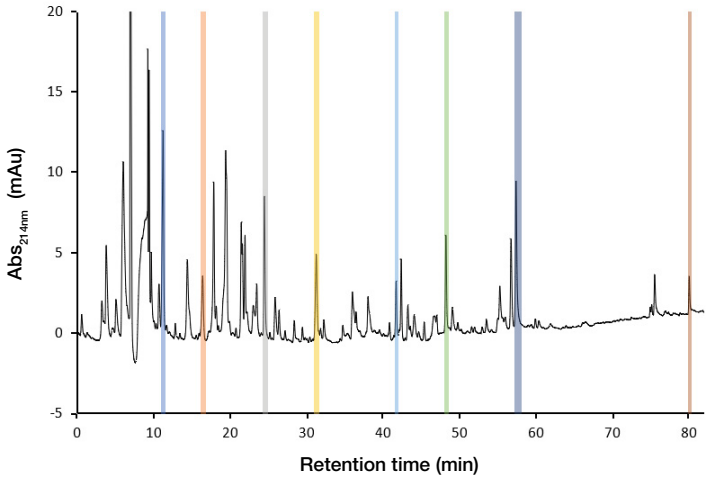

Figure 2. Representative LC-UV chromatogram for a 1 pmol injection of BSA protein digest onto a 75 µm × 50 cm PepMap Neo column. The 8 peaks selected for evaluation are highlighted.

Results and discussion

The Vanquish Neo UHPLC system was continuously run using a single 75 µm × 50 cm PepMap Neo column for a period of 6 months. The BSA protein digest was separated using a classic 90-minute nano-flow gradient (100 minute method including column washing) typical for bottom-up proteomic experiments.

The peak resolution of BSA peptides afforded by the separation, permitted the evaluation of chromatographic parameters for 8 selected peaks using UV detection (Figure 2). Peptide retention times were stable across all 1600 injections (Figure 3). The retention time standard deviation was below 0.3 min over the entire 6 months period for each set of 100 injections.

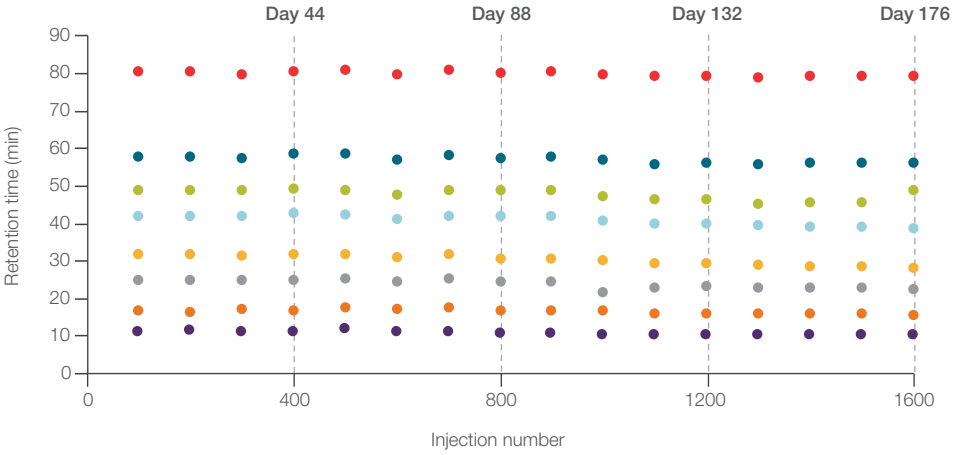

Figure 3. The retention time for 8 selected peptides from 1,600 injections of BSA protein digest over 176 days (approximately 6 months). Retention time values are the means per set of 100 injections.

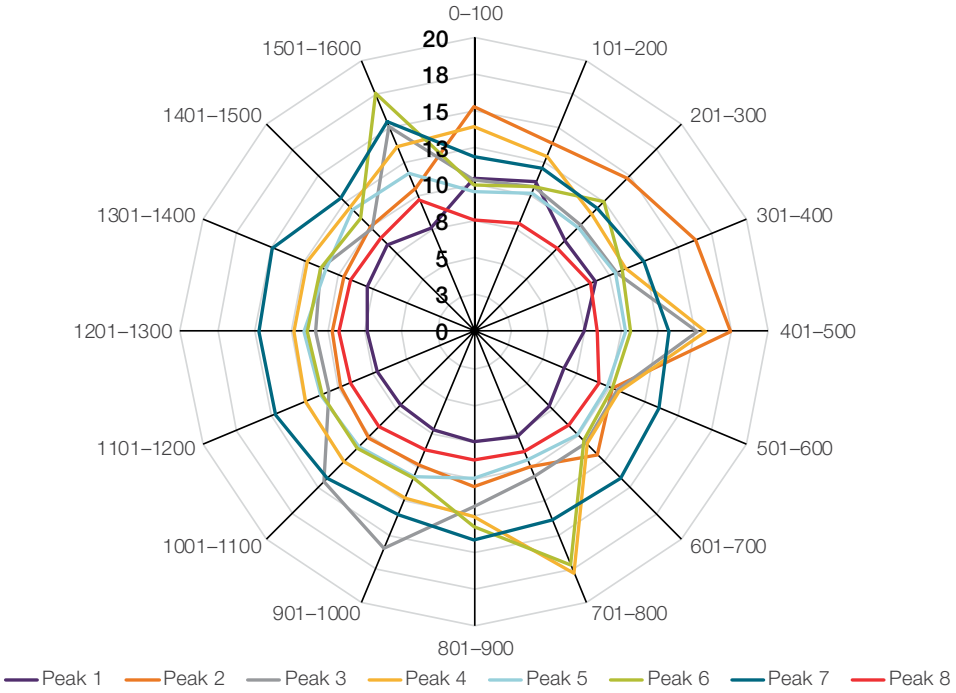

Figure 4. Mean values for FWHM of 8 selected peptides over 1,600 replicate injections of BSA protein digest. Displayed are mean peak widths (seconds) per 100 injections for each of the selected peptides.

FWHM was also assessed for the same set of peptides as a marker of resolution consistency and robustness (Figure 4). Consistent FWHM for the 8 selected peptides was observed throughout the study. There was no trend towards increased peak width associated with any of the peptides studied. Instead a "random" variation of FWHM can be seen for some of the peptides measured which can be attributed to overlapping peaks which in some instances could not be resolved by UV. The hallmarks of reproducible gradient delivery and separation column robustness observed for the duration of the study can be attributed to various system features designed to prolong column lifetime and maintain optimal separation performance such as the controlled flow ramping employed during fast sample loading and fast column equilibration and Thermo Scientific™ SmartInject functionality that reduces the pressure shock on the column material. Additionally, a filter frit integrated into the needle seat prevents sample debris from accumulating on the head of the column, further improving method robustness and column lifetime. The robustness of the separations is also reflected by the very stable column back pressure which varied by less than 25 bar over the 1600 injection/6 month analysis period (Figure 5).

Conclusions

Bottom-up proteomics LC-MS analyses using long separation columns and nano-flow rates have long been established as the mainstay of proteomics research. However, general concerns regarding robustness and reproducibility of the methodology combined with the technical challenges considered inherent to nanoLC, have prevented its widespread adoption into "routine" applications, despite the potential benefits including increased sensitivity and reduced solvent consumption. This proof-of-principle study demonstrates that the Vanquish Neo UHPLC system and the PepMap Neo columns deliver levels of chromatographic robustness and reproducibility required for long-term trouble-free nanoLC operation under maximum performance and pressure conditions. The unprecedented levels of sensitivity and eluent saving efficiency afforded by the technique came at no cost to either instrument robustness or method ruggedness. Taken together, these data are evidence of the reliability and performance capabilities of modern low-flow UHPLC systems and consumables.

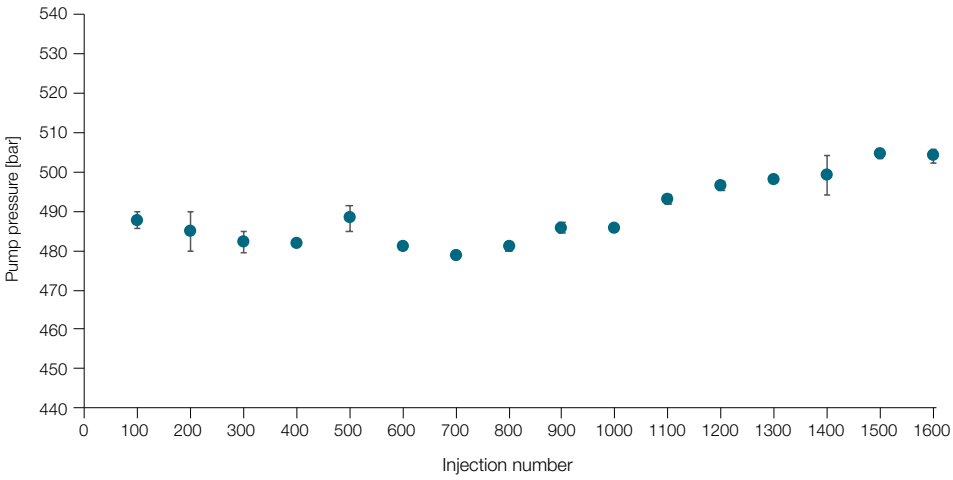

Figure 5. Column back pressure measured 1 minute after the start of the gradient at a flow rate of 300 nL/min and 50 °C column temperature on a 75 µm × 50 cm PepMap Neo column. Results are mean values per 100 injections ± S.D.

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