LC-MS

TECHNICAL NOTE

Vanquish Neo UHPLC system-to-system reproducibility ensures consistent and reliable results in nanoLC-MS proteomics

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Keywords

Vanquish Neo UHPLC system, Orbitrap Exploris 240, EASY-Spray PepMap Neo column, System-to-System reproducibility, nanoLC-MS

Goal

Demonstrate the Thermo Scientific™ Vanquish™ Neo UHPLC system-to-system reproducibility based on peak properties and proteomic data metrics for nanoLC-MS analysis.

Introduction

NanoLC-MS analysis employing long, narrow-bore columns and long gradients is well established as the gold standard for bottom-up discovery proteomics applications. Aside from the technical challenges associated with nanoLC, concerns remain around the day-to-day and system-to-system reproducibility of results. High system-to-system reproducibility is essential to generate global data sets that can be jointly processed and to gain statistically significant insights for biomarker discovery and validation, characterization of cell lines, and drug development.

We investigated the intra- and inter-system variability for typical nanoLC-MS applications using the new Vanquish Neo UHPLC system. Both short (20 min) and standard bottom-up proteomics (100 min) nanoLC methods on a 75 µm I.D. × 50 cm, 2 µm Thermo Scientific™ EASY-Spray™ PepMap™ Neo column, were used to compare results for six different systems. Reproducibility was assessed based on the retention time stability of individual peptides and the number of protein and peptide identifications collected and processed under standardized conditions.

Materials and methods

Sample preparation

Thermo Scientific™ Cytochrome C protein digest standard (1.6 nmol/vial, PN 161089) was reconstituted by adding 200 µL of 5% Acetonitrile/95% Water (v/v) with 0.1% formic acid (FA) in water. The vial was subsequently sonicated for 15 min. The sample was then diluted 8 fold using 0.1% FA in water to yield a final sample concentration of 1 pmol/µL of Cytochrome C protein digest.

Thermo Scientific™ Pierce™ HeLa Digest/PRTC Standard (A47996, 10 µg/vial) was reconstituted by adding 50 µL of 0.1% formic acid (FA) in water. The vial was subsequently sonicated for 2 min, followed by multiple sample aspiration and release cycles with a pipette to dissolve it completely. The final sample concentration was 200 ng/µL HeLa digest with 100 fmol/µL PRTC.

Consumables

- Fisher Scientific™ LC-MS grade Water with 0.1% Formic Acid (P/N LS118500)
- Fisher Scientific™ LC-MS grade 80% Acetonitrile with 0.1% Formic Acid (P/N LS122500)
- Fisher Scientific™ LC-MS grade Formic Acid (FA) (P/N A117-50)
- Fisher Scientific™ LC-MS grade Isopropanol (P/N A461-212)

LC-MS analyses were performed on Vanquish Neo UHPLC systems connected to the same Thermo Scientific™ Orbitrap Exploris™ 240 mass spectrometer (Figure 1). The LC system configuration, fluidics, and consumables used to set up the Vanquish Neo UHPLC systems in order to run the direct injection workflow are given in Table 1.

Table 1. Vanquish Neo UHPLC system, 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 and ship kit	1	VN-S10-A-01
Vanquish display (required)	1	6036.1180
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
EASY-Spray PepMap Neo column 75 µm × 500 mm, 2 µm, 1500 bar	1	ES75500PN

Figure 1. Vanquish Neo UHPLC system and Orbitrap Exploris 240 mass-spectrometer.

LC solvents

The solvents required to run the nanoLC-MS applications are described in Table 2.

Table 2. Solvents required for Vanquish Neo UHPLC system operation

	Solvent	Composition
Binary Pump N	Mobile phase A	H_2O with 0.1% FA
	Mobile phase B	80/20 (%, v/v) ACN/ H_2O with 0.1% FA
Split Sampler NT Metering Device	Weak wash liquid	H_2O with 0.1% FA
	Strong wash liquid	80/20 (%, v/v) ACN/ H_2O with 0.1% FA
Split Sampler NT Wash Port	Weak wash liquid	H_2O with 0.1% FA
	Strong wash liquid	80/20 (%, v/v) ACN/ H_2O with 0.1% FA
Binary Pump N and Split Sampler NT	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 separation gradients for the two LC methods are described in Table 4.

Table 3. Vanquish Neo UHPLC system parameters

	Parameter	Value
Sample loading	Fast loading	Enabled
	Mode	Combined Control
	Flow	5 µL/min
	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	3.0 µL/s
	Vial bottom detection	Enabled
Column equilibration	Fast equilibration	Enabled
	Mode	Combined Control
	Flow	5 µL/min
	Pressure	1500 bar
	Equilibration factor	2
Temperature	EASY-Spray column	40 °C
	Autosampler	7 °C

*System default values

Table 4. Separation gradients for 20 min and 100 min nanoLC-MS methods

20 min method

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.35	1
0.1	0.1	0.35	4
14.1	14	0.35	50
Column wash phase			
14.2	0.1	0.35	99
20	5.8	0.35	99

100 min method

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.3	1
0.1	0.1	0.3	6
60.1	60	0.3	20
90.1	30	0.3	35
Column wash phase			
91.1	1	0.3	99
100	8.9	0.3	99

MS acquisition parameters

MS data were acquired using an Orbitrap Exploris 240 mass spectrometer in Full Scan or data-dependent acquisition (DDA) mode under Thermo Scientific™ Chromeleon™ 7.2.10 MUd Chromatography Data System (CDS) control. Global MS method parameters are given in Table 5, method specific parameters are in Table 6.

Table 5. Global MS Scan Parameters

Category	Property	Common setting
Method Settings	Application Mode	Peptide
Ion Source	Positive Ion (V)	1900
	Ion Transfer Tube Temp (°C)	275
MS Global Settings	Infusion Mode	Liquid Chromatography
	Expected LC Peak Width (s)	15
	Advanced Peak Determination	TRUE
	Default Charge State	2
	Internal Mass Calibration	Off
Full Scan	Scan Range *(m/z)*	375–1200
	AGC Target	Custom
	RF Lense (%)	80
	Data Type	Profile
	Polarity	Positive
	Source Fragmentation	Disabled
MIPS	Monoisotopic peak determination	Peptide
	Relax restrictions when too few precursors are found	TRUE
Charge State	Include charge state(s)	2–5
	Include undetermined charge states	FALSE
Dynamic Exclusion	Perform dependent scan on single charge state per precursor only	TRUE
ddMS²	Multiplex Ions	FALSE
	Isolation window *(m/z)*	2
	Collision Energy Type	Normalized
	HCD Collision Energy (%)	26
	Scan range Mode	Define First Mass
	First Mass *(m/z)*	120
	Normalized AGC Target (%)	50
	Data Type	Centroid

Table 6. Specific MS method settings for each LC method

Category	Property	20 min method Full Scan	100 min method DDA
Method Settings	Method Duration (min)	20	100
Full Scan	Orbitrap Resolution	120,000	120,000
	RF Lens	80	80
Intensity	Intensity Threshold	—	1.0E+04
Dynamic Exclusion	Exclusion duration (s)	—	45
Data Dependent	Number of Dependent Scans	—	40
ddMS²	Orbitrap Resolution	—	15,000
	Maximum Injection Time (ms)	—	12

Data processing and analysis

Cytochrome C protein digest and PRTC peptide peak properties were extracted using Chromeleon CDS. HeLa digest proteomics data were processed using 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 the peptide and the protein level.

Results and discussion

Intra-system retention time reproducibility

There is an increasing requirement for high retention time reproducibility in nanoLC-MS proteomics workflows. This is particularly true for label-free quantitative LC-MS analysis where peptide retention times can be used to increase identification confidence and to compare results collected using different LC-MS setups. It is also a powerful tool for increasing the number of quantifiable peptides via the matching of peaks between runs. Intra-system retention time reproducibility was assessed by comparing replicate 1 pmol Cytochrome C peptide digest separations carried out on 75 µm × 500 mm EASY-Spray PepMap Neo columns for 6 different Vanquish Neo UHPLC systems. A 20 min method (Table 4) was employed with Full Scan data acquisition carried out on an Orbitrap Exploris 240 mass spectrometer. The results for 4 replicate injections on 6 systems showed excellent retention time precision with <0.2% RSD for all peptides selected for data processing. Mean retention times (Figure 2A) and relative standard deviation (RSD), % (Figure 2B) for each peptide across all 6 Vanquish Neo UHPLC systems are given below.

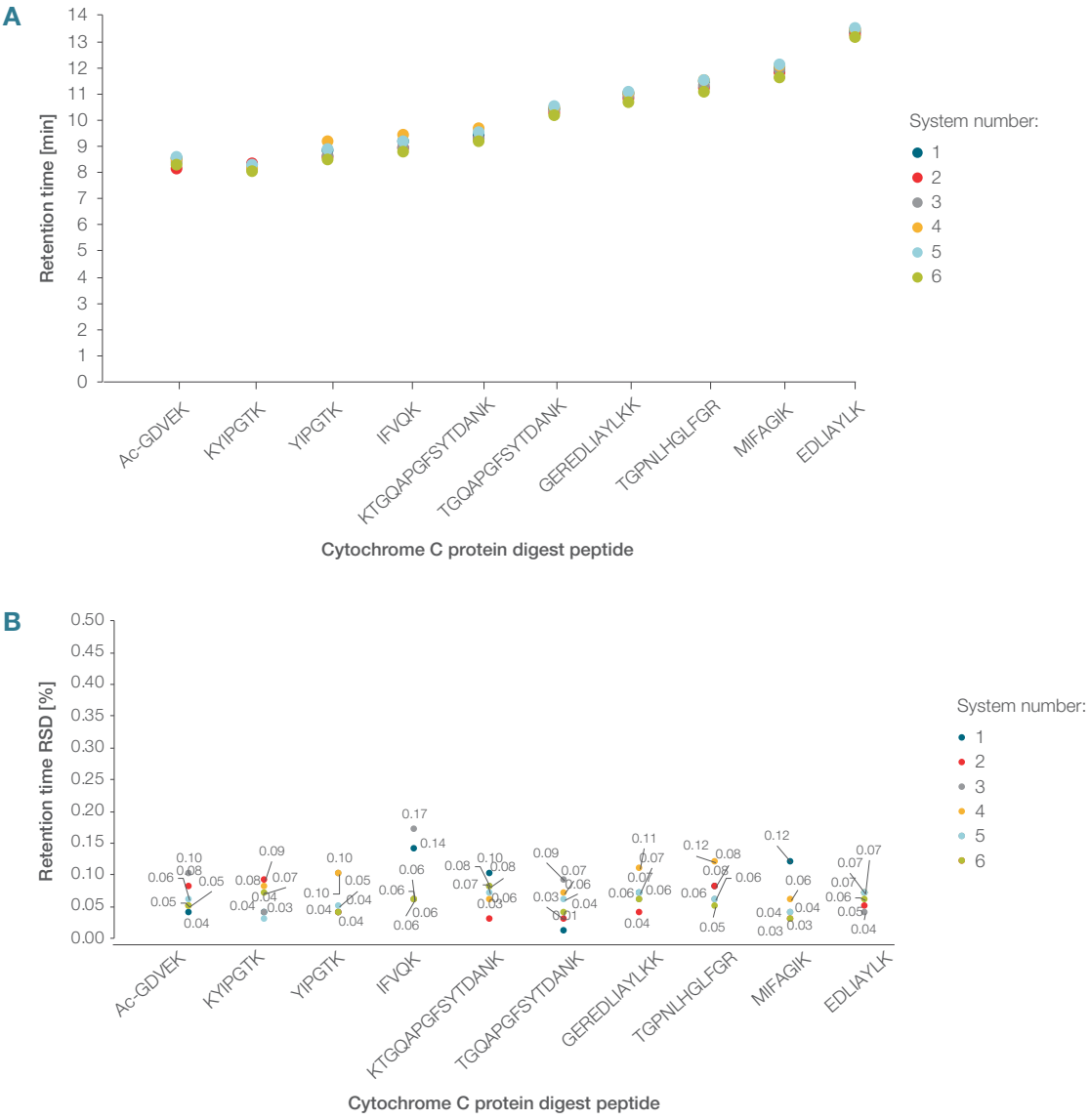

Figure 2. Mean retention times (A) and RSD, % values (B) for selected Cytochrome C protein digest peptides separated on 6 Vanquish Neo UHPLC systems using a 20 min nanoLC method; 4 replicate injections were carried out for each system.

Vanquish Neo UHPLC system-to-system reproducibility according to peptide peak properties

Inter-system retention time reproducibility was assessed for 6 Vanquish Neo UHPLC systems with Cytochrome C protein digest using the 20 min method (Figure 3A) as well as HeLa cell protein digest spiked with PRTC peptide standard using a 100 min nanoLC-MS method (Figure 3B).

Retention time standard deviation between systems varied between 0.1 and 0.25 minutes for the 20 min method and were below 2 minutes for the 100 min method for all studied peptides across the wide hydrophobicity range.

The reproducibility of PRTC peptide retention times was matched by the high reproducibility observed for other peak properties such as area, height, and FWHM where RSDs were at or below 15% for all PRTC peptides across all systems (Table 7).

Table 7. Variation in peak properties: Area, Height and FWHM, for each of the PRTC peptides separated using the 100 min method; values are RSD, % across 6 Vanquish Neo UHPLC Systems with 4 injections per system

PRTC Peptide	Area RSD [%]	Height RSD [%]	FWHM RSD [%]
SSAAPPPPPR	8.0	10.1	10.0
HVLTSIGEK	8.0	10.7	11.8
GISNEGQNASIK	6.8	7.1	8.5
IGDYAGIK	5.7	6.7	9.7
DIPVPKPK	8.4	7.1	4.6
TASEFDSAIAQDK	6.4	7.2	9.3
SAAGAFGPELSR	6.4	7.3	3.4
ELGQSGVDTYLQTK	6.5	8.2	5.9
GLILVGGYGTR	12.1	11.8	13.9
SFANQPLEVVYSK	6.0	6.6	7.8
GILFVGSGVSGGEEGAR	5.6	6.9	8.7
LTILEELR	7.7	9.4	10.7
NGFILDGFPR	5.5	6.5	2.8
ELASGLSFPVGFK	4.9	7.4	8.4
LSSEAPALFQFDLK	11.3	6.8	15.3

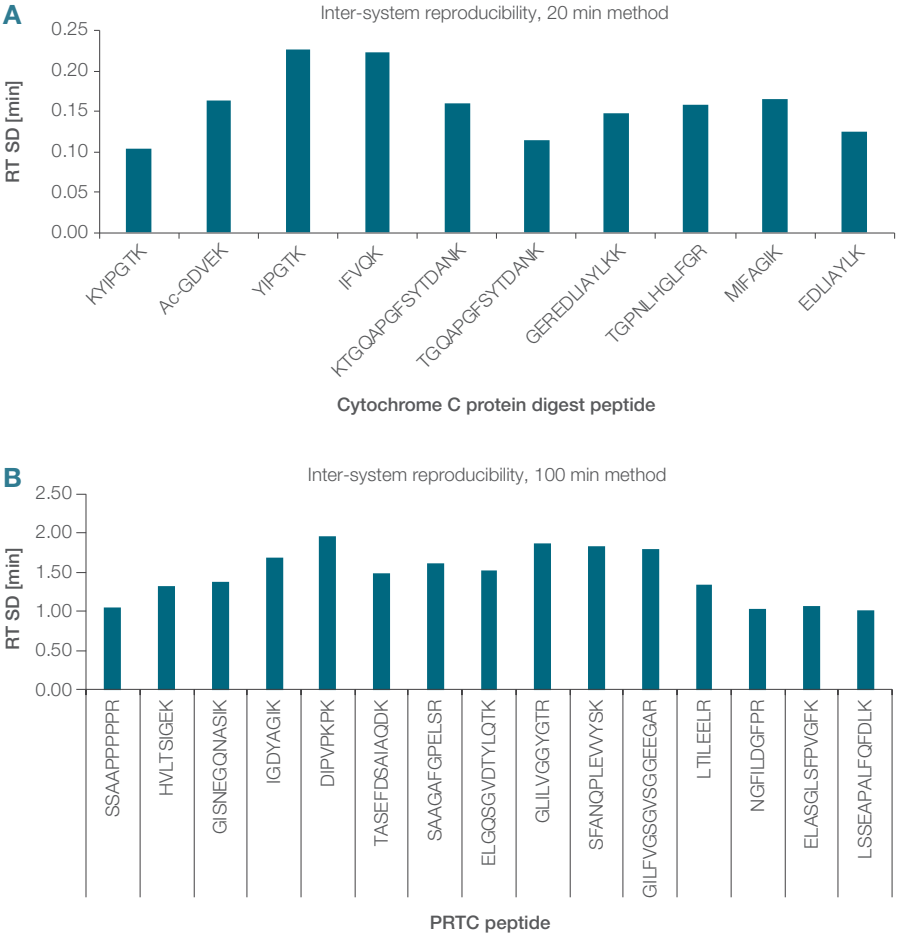

Figure 3. The retention time standard deviation of Cytochrome C peptides using a 20 min method (A) and of PRTC peptides using a 100 min method (B) for 6 individual Vanquish Neo UHPLC systems; 4 replicate injections for each system.

Vanquish Neo UHPLC system-to-system reproducibility permits consistent proteomics data acquisition

High-quality, reproducible nanoLC-MS data generation across systems and sites is crucial to the success of routine quantitative proteomics applications. System-to-system reproducibility according to peptide and protein IDs was evaluated using the 100 min method with 200 ng injections of a HeLa cell protein digest under standardized MS acquisition conditions (Figure 4).

On average 33,000 peptides from 4,400 protein groups could be identified with a single-shot nanoLC-MS analysis. As little as 4.1% variation in peptide groups and 2.2% in protein groups was observed for the 6 Vanquish Neo UHPLC systems coupled to an Orbitrap Exploris 240 mass spectrometer.

Conclusions

NanoLC-MS has long been considered the mainstream methodology in proteomics-based research due to its high sensitivity. While increased ease-of-use and robustness are essential to its widespread adoption, the transition from academic research to industrial or clinical research applications is equally dependent upon achieving the highest levels of both intra- and inter-system reproducibility.

This study demonstrates how the combined power of the Vanquish Neo UHPLC system, accompanying PepMap Neo columns and the latest HRAM Orbitrap mass spectrometers deliver the level of system-to-system reproducibility required to meet the demands of large cohort and multi center studies. In particular, the capacity of the Vanquish Neo UHPLC system to deliver standardized, rugged, and reproducible analytics will help foster the adoption of nanoLC-MS for large sample cohorts analysis.

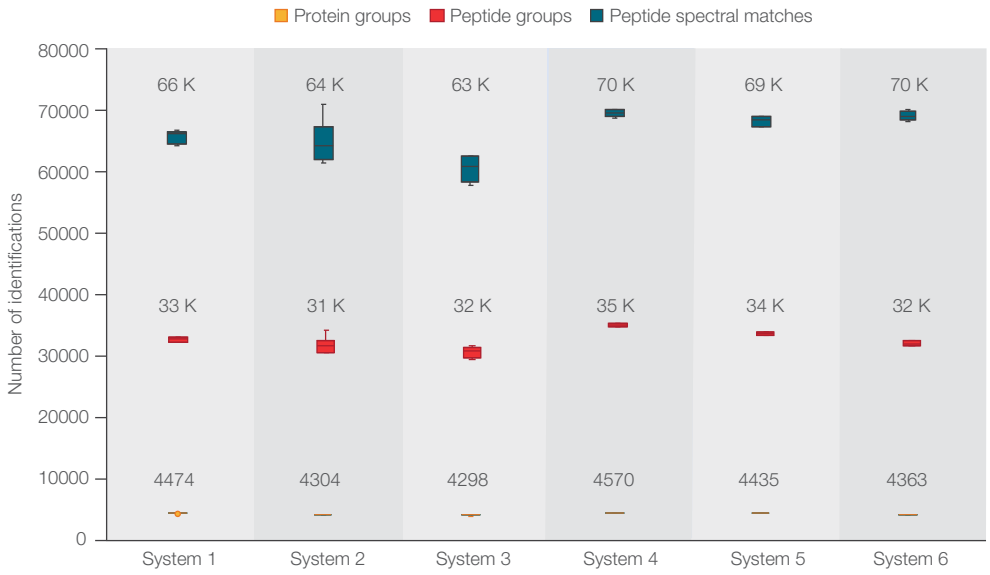

Figure 4. Comparison of 200 ng HeLa protein digest profiling with 6 different Vanquish Neo UHPLC systems coupled to an Orbitrap Exploris 240 mass spectrometer. For the separation, 75 µm × 50 cm, 2 µm EASY-Spray PepMap Neo columns, a 90 min gradient (100 min method), 300 nL/min flow rate, direct injection, and 1500 bar sample loading and column equilibration were used.

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