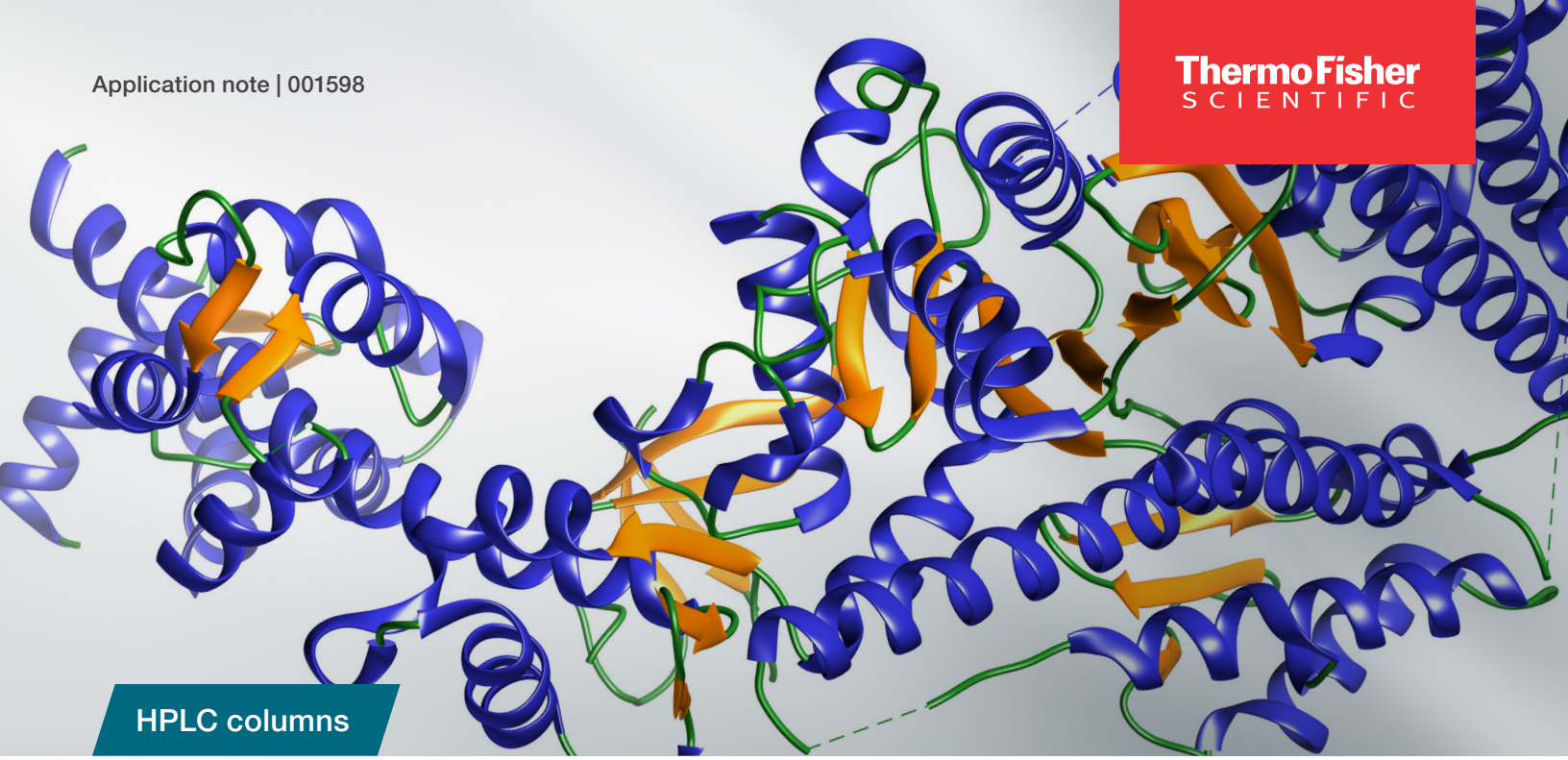
Application note | 001598



HPLC columns

The unique separation performance of µPAC Neo HPLC columns gives increased coverage in single-shot nanoLC-MS bottom-up proteomic research

Authors

Jeff Op de Beeck[1], Natalie Van Landuyt[1], Xuefei Sun[2], Yuan Lin[2], Keiryn L. Bennett[1] Tabiwang Arrey[3], Julia Kraegenbring[3], Paul Jacobs[1]; [1]Thermo Fisher Scientific, Ghent, Belgium; [2]Thermo Fisher Scientific, Sunnyvale, CA, USA; [3]Thermo Fisher Scientific, Bremen, Germany

Keywords

µPAC Neo HPLC column, Vanquish Neo UHPLC system, Orbitrap Exploris 240 mass spectrometer, EASY-Spray bullet emitter, bottom-up proteomics, limited sample proteomics, high-throughput proteomics, deep proteome coverage, HeLa protein digest standard, column-to-column reproducibility, column robustness

Goal

To demonstrate the performance of Thermo Scientific™ µPAC™ Neo HPLC Columns across a variety of 'bottom-up' proteomic operational ranges using the Thermo Scientific™ Vanquish™ Neo UHPLC System coupled to the Thermo Scientific™ Orbitrap Exploris™ 240 Mass Spectrometer.

Introduction

From the inception of 'bottom-up' proteomic profiling, the separation resolution that can be achieved when reversed-phase nanoLC is coupled to high-resolution mass spectrometry has been one of the critical components propelling improvements in the field. Analogous to the standard flow liquid chromatography sector, where silica particle dimensions have been continuously reduced to reach the goal of increased separation power, nanoLC column formats have seen a similar evolution throughout the years.[1] 75 µm I.D. columns packed with sub-2 µm fully-porous silica particles are currently the gold standard in 'bottom-up' proteomic research. To maximize peak resolution and provide maximal optimal separation of a diverse array of tryptic peptides with different lengths and properties, typical columns are to some extent longer than those used in other application areas. Columns up to 75 cm, and theoretically capable of resolving close to 1,000 chromatographic peaks, are no exception.[2] However, this has a serious impact on operational flexibility and the HPLC pump pressures required to run such experiments.

An alternative to packed bed (and monolithic) column technology is the microfabricated pillar array columns (µPAC™) which were introduced as an innovative technology that enables high peak capacity separations at moderate LC pump pressures. Through the implementation of lithographic pattern transfer and deep reactive ion etching (DRIE) into silicon wafers, separation channels can be manufactured that contain micrometer-sized silicon features that are perfectly positioned according to a pre-defined design. The introduction of the perfectly ordered separation beds eliminates any Eddy dispersion originating from heterogeneous flow paths through the column and increases column permeability.[3] From 2017, the first generation of pillar array based nanoLC columns with lengths ranging from 50 to 200 cm saw gradual adoption into the field of 'bottom-up' proteomics. To expand the µPAC nanoLC column portfolio and to further exploit the potential of this technology, µPAC Neo HPLC Columns have been developed where critical dimensions have been reduced by a factor of 2. Compared to the first-generation columns, this provides a net gain in separation resolution of approximately 1.4 and enables full resolution of more chromatographic peaks over a shorter analysis time. In addition, the µPAC Neo HPLC columns have Thermo Scientific™ nanoViper™ Fingertight Fittings integrated on both sides of the column. This enables easy and reproducible connection whilst minimizing the introduction of dead volumes. Within the µPAC Neo column portfolio, three options are available. For the analysis of low sample amounts that require maximum sensitivity, a non-porous 50 cm column is recommended. High-to-medium throughput analyses of more conventional nanoLC protein digest amounts will benefit from the increased interaction surface provided by the superficially porous 50 cm Neo column. And finally, even higher sample amounts and comprehensive 'single-shot' analyses will benefit from the unrivaled peak capacity offered by the superficially porous 110 cm µPAC Neo column.

Here, we present an extensive evaluation of the three µPAC Neo columns with the goal to guide users in selecting the appropriate column when designing nanoLC-MS experiments. By coupling the µPAC Neo columns to a Thermo Scientific™ Vanquish™ Neo UHPLC System and collecting MS/MS data in data dependent acquisition (DDA) mode on an Orbitrap Exploris 240 mass spectrometer, we provide a clear evaluation of the proteome coverage that can be obtained using µPAC Neo columns. The high permeability of the columns provides operational flexibility as higher flow rates can be used during sample loading and equilibration. Flow rates up to 750 nL/min can be achieved within the allowed pressure limit of 450 bar. Additional features such as excellent column-to-column reproducibility and data consistency throughout the lifetime of the column were evaluated on twelve 50 cm µPAC Neo columns sourced from three production batches.

Experimental materials and methods

Sample preparation

The Thermo Scientific™ Pierce™ HeLa Protein Digest Standard was reconstituted and diluted to three different final concentrations. For the low-load experiments (1 to 20 ng on column), 20 µg was reconstituted by adding 400 µL of 0.1% formic acid (FA) in water. The vial was subsequently sonicated for 2 min, followed by aspiration and release 10-times with a pipette to fully solubilize the sample. 20 µL was then transferred to a vial containing 180 µL of 0.1% FA in water to obtain the final concentration of 10 ng/µL. Sonication and mixing were again performed prior to placing the vial into the autosampler. For the medium load experiments (50 to 200 ng on column), 20 µg was reconstituted by adding 100 µL of 0.1% FA in water to obtain a final concentration of 200 ng/µL. After sonication and mixing, the content was first transferred to a 0.2 mL vial and then the vial was placed in the autosampler. For the high load experiments (500 to 4,000 ng on column), 20 µg was reconstituted by adding 20 µL of 0.1% FA in water to obtain a final concentration of 1,000 ng/µL. After sonication and mixing, the contents were first transferred to a 0.2 mL vial, and then the vial was placed in the autosampler. Thermo Scientific™ Dionex™ Cytochrome C Digest was reconstituted to a stock concentration of 8 pmol/µL by adding 200 µL of 0.05% trifluoroacetic acid (TFA) in 95% water and 5% acetonitrile. After sonication and mixing, the stock solution was diluted to a final concentration of 125 fmol/µL in 0.05% TFA.

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 LS122500)
- Fisher Scientific™ LC-MS grade Formic acid (P/N A117-50)
- Fisher Scientific™ LC-MS grade Trifluoroacetic acid (TFA) (P/N A116-50)
- Fisher Scientific™ LC-MS grade Isopropanol (P/N A461-212)
- Thermo Scientific™ Pierce™ HeLa Protein Digest Standard (P/N 88328)
- Thermo Scientific™ Dionex™ Cytochrome C Digest (P/N 161089)
- Fluidics and columns used to configure the Vanquish Neo UHPLC system for direct injection and coupling to the Orbitrap Exploris 240 mass spectrometer are listed in Table 1.

Table 1. Fluidics and accessories for the direct injection workflow.

Description	#	Part number
Vial and cap 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™ EASY-Spray™ Nano Emitter, bullet type without transfer line	3	ES993
50 cm µPAC Neo Low-load HPLC column	1	COL-lolo050NeoB
50 cm µPAC Neo HPLC column	12	COL-nano050NeoB
110 cm µPAC Neo HPLC column	1	COL-nano110NeoB

LC solvents

The solvents used in the nanoLC-MS experiments are listed in Table 2.

Table 2. Solvents used for instrument operation.

Module	Property	Setting
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
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
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 metering device	Rear seal wash buffer	25/75 (v/v) H_2O/isopropanol with 0.1% FA

LC method parameters

Necessary column specifications for the three columns on the Vanquish Neo UHPLC system and the parameters used for sample aspiration, loading, and column equilibration are shown in Table 3. Typical LC gradient profiles for each column are listed in Table 4.

Table 3. LC column specifications on the VSC UI and LC method parameters.

Category	Parameter	50 cm µPAC Neo Low-load column	50 cm µPAC Neo column	110 cm µPAC Neo column
Separation column specifications	Inner diameter (µm)	75	75	75
	Length (cm)	50	50	150
	Void volume (µL)	1.48	1.48	4.44
	Maximum pressure (bar)	450	450	450
	Maximum flow (µL/min)*	100	100	100
	Maximum temperature	45°C	60°C	60°C
	Maximum pressure change up (bar/min)	1,000	1,000	1,000
	Maximum pressure change down (bar/min)	1,000	1,000	1,000
Sample loading	Fast loading	Enabled	Enabled	Enabled
	Mode	PressureControl	PressureControl	PressureControl
	Flow	—	—	—
	Pressure (bar)	400	400	400
	Loading volume (µL)	1.5	1.5	1.5
Column equilibration	Fast equilibration	Enabled	Enabled	Enabled
	Mode	PressureControl	PressureControl	PressureControl
	Flow	—	—	—
	Pressure (bar)	400	400	400
	Equilibration factor	1.5	1.5	1.5
Temperature	Column compartment temperature	40°C	50°C	50°C
	Autosampler temperature	7°C	7°C	7°C

*Maximum flow is set at 100 µL/min during sample loading and column equilibration, this can only be achieved when sample loading and column equilibration is performed in PressureControl mode. For CombinedControl or FlowControl mode, 0.8 µL/min is advised for all columns.

Table 4. Typical LC methods.

50 cm µPAC Neo Low-load column

30 min method—classic

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.25	0
0.1	0.1	0.25	1
22.1	22	0.25	17.5
30.1	8	0.25	35
Column wash phase			
30.6	0.5	0.25	99
38	7.4	0.25	99

22 min method—throughput

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.75	0
0.1	0.1	0.75	1
2	6.4	0.75	6
2.1	0.1	0.25	6.1
12.1	10	0.25	17.5
15.6	3.5	0.25	35
Column wash phase			
16.1	0.5	0.25	99
22	5.9	0.25	99

50 cm µPAC Neo column

60 min method—classic

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.25	1
0.1	0.1	0.25	2
44.1	44	0.25	22.5
60.1	16	0.25	45
Column wash phase			
60.6	0.5	0.25	99
68	7.4	0.25	99

22 min method—throughput

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.75	1
0.1	0.1	0.75	4
2	1.9	0.75	10
2.1	0.1	0.25	10.1
12.1	10	0.25	22.5
15.6	3.5	0.25	45
Column wash phase			
16.1	0.5	0.25	99
22	5.9	0.25	99

110 cm µPAC Neo column

150 min method—classic

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.25	1
0.1	0.1	0.25	2
110.1	110	0.25	22.5
150.1	40	0.25	45
Column wash phase			
150.6	0.5	0.25	99
175	24.4	0.25	99

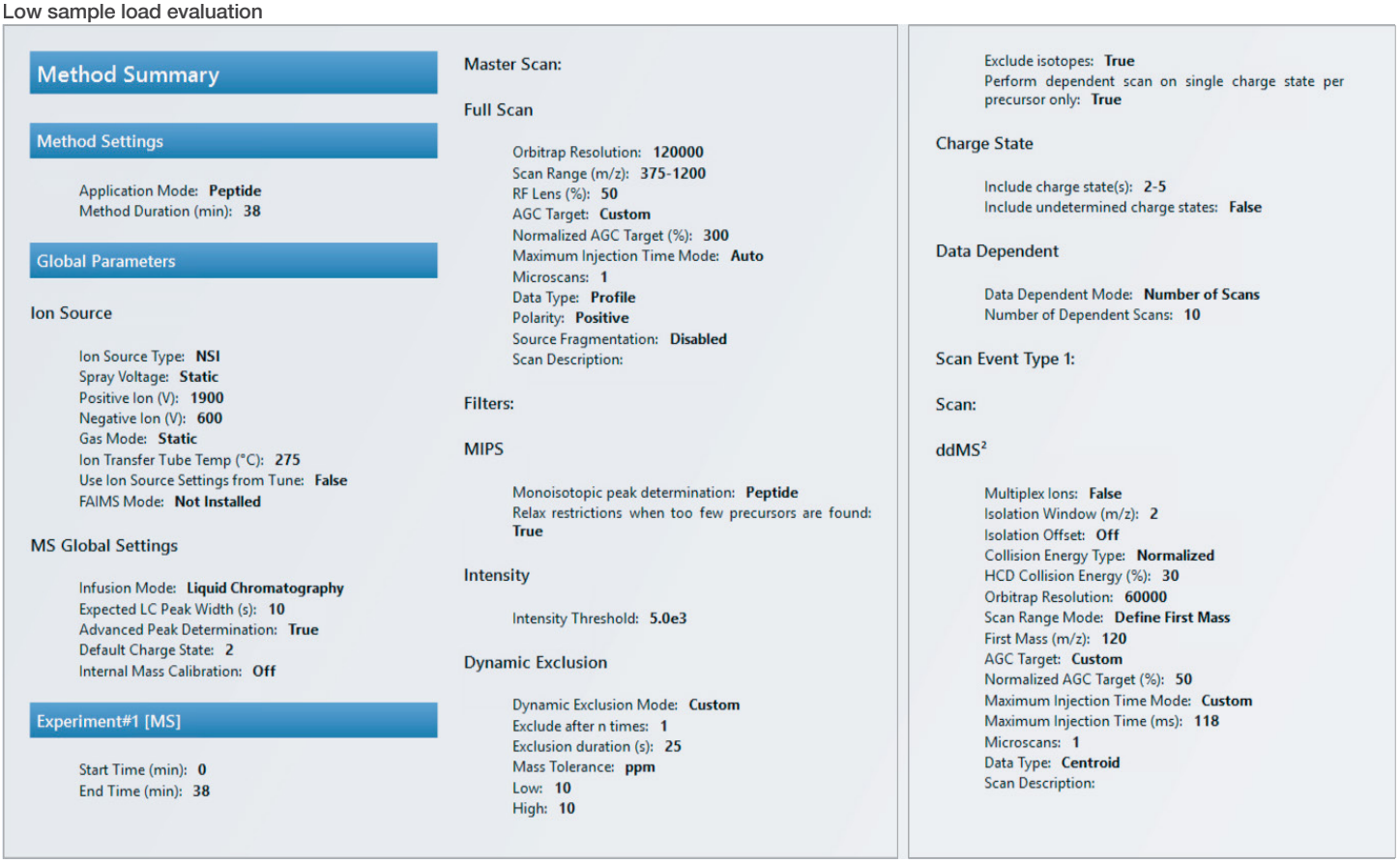
47 min method—throughput

Time (min)	Duration (min)	Flow rate (µL/min)	%B
Gradient separation phase			
0	0	0.75	1
0.1	0.1	0.75	2
6.5	6.4	0.75	15
6.6	0.1	0.25	15.1
17	10.4	0.25	22.5
27	10	0.25	45
Column wash phase			
27.5	0.5	0.25	99
47	19.5	0.25	99

Data acquisition and processing

MS data were acquired on an Orbitrap Exploris 240 mass spectrometer in DDA mode. Depending on the operation range (sample load and gradient time), MS settings were varied to obtain optimal proteome coverage. MS data acquisition parameters for low, medium and high sample load evaluation are shown in Figure 1. MS/MS spectra from raw data were imported into Thermo Scientific™ Proteome Discoverer™ software version 3.0 and processed using a standard CHIMERYS™ percolator workflow extended with the apQuant node for FWHM determination.[4,5] The false discovery rate was set to below 1% at the peptide and protein level. The match-between-runs (MBR) was disabled.

Low sample load evaluation



Medium and high sample load evaluation

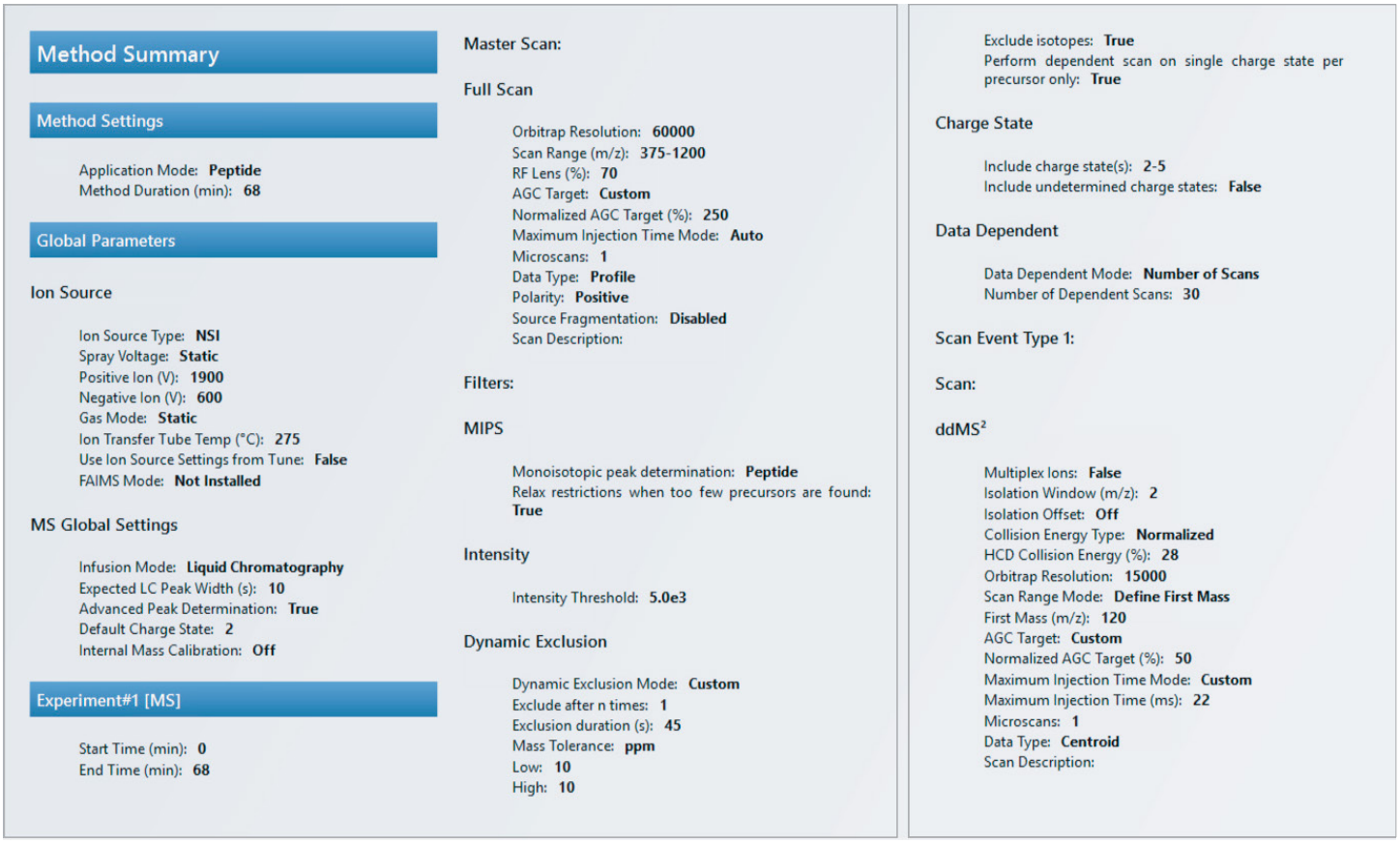


Figure 1. Examples of Orbitrap Exploris 240 mass spectrometer parameters for the analysis of low and medium/high sample amounts.

Results and discussion

Operating pressure/permeability

As a result of the high permeability associated with ordered stationary phase support structures, µPAC Neo HPLC columns can be used over a wide flow range (100–750 nL/min). At conventional nanoLC flow rates (200–400 nL/min), the pressure typically ranges from 75 to 200 bar and is comparable across all three columns of the µPAC Neo column portfolio (Figure 2). Despite the increased separation length of the 110 cm column, the pump pressure required to generate a specific flow rate is equivalent to the pressure required for the 50 cm column. This is attributed to the increased separation channel cross section (etching depth of 30 µm instead of 16 µm, Figure 3), however, the deeper etching leads to a significant increase in column volume from 1.5 µL for the 50 cm column to approximately 4.5 µL. Thus, sample elution and column equilibration require more time for these longer columns. For the 50 cm columns, 3 min are typically required for sample loading and column equilibration at the maximum pressure of 400 bar (column volume 1.5 µL); however, this can take up to 9 min for the 110 cm column. Thus, the 110 cm column is less efficient for shorter analyses.

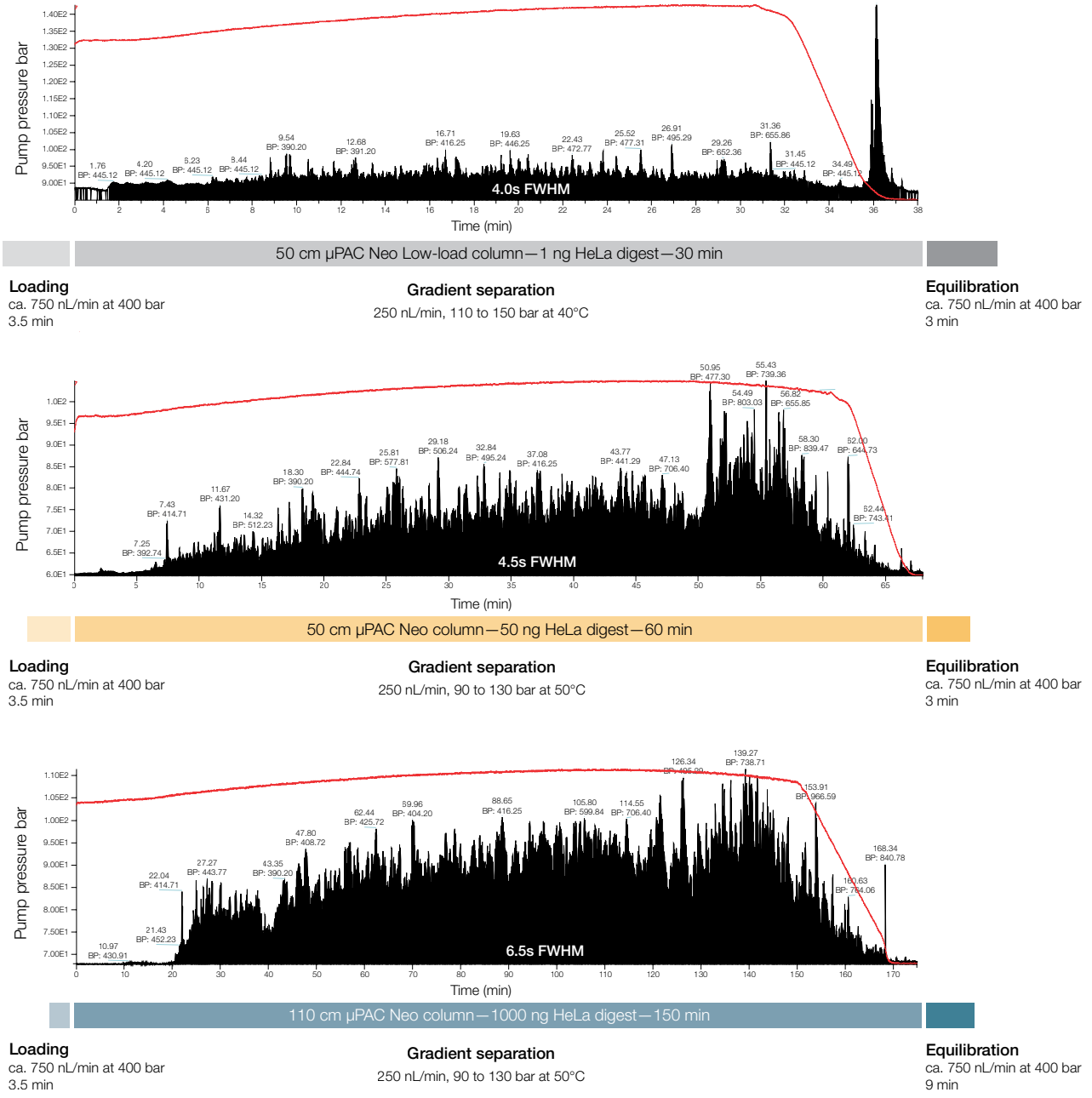


Figure 2. Typical chromatograms were obtained on the 50 cm µPAC Neo low-load column, 50 cm µPAC Neo column, and 110 cm µPAC Neo column, respectively. Pressure profiles are overlaid in red. The relative contribution of the sample loading and column equilibration steps are indicated in the bars below the images.

	50 cm µPAC Neo Low-load column	50 cm µPAC Neo column	110 cm µPAC Neo column
Pillar shape	Cylindrical	Cylindrical	Cylindrical
Pillar diameter (µm)	2.5	2.5	2.5
Interpillar distance (µm)	1.25	1.25	1.25
Channel width (µm)	180	180	180
Channel depth (µm)	16	16	30
Column length (cm)	50	50	110
Column volume (µL)	1.5	1.5	4.5
Surface morphology	Non-porous	Core-shell	Core-shell
Porous layer thickness (nm)	—	300	300
Pore size range (A)	—	100–300	100–300
Surface functionalization	C18 + HMDS	C18 + HMDS	C18 + HMDS
Flow rate range (nL/min)	100–750	100–750	100–750
Loadability range (ng)	0–10	10–500	500–2000

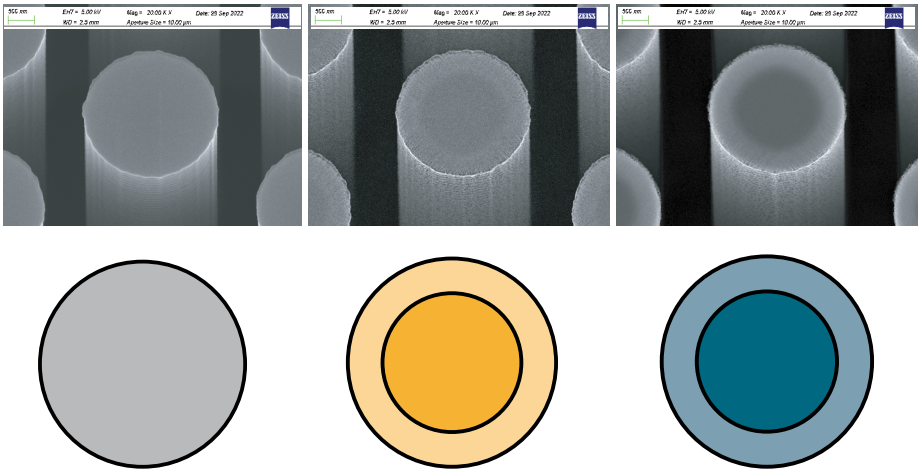


Figure 3. Column specifications for the three µPAC Neo HPLC columns. Scanning electron microscopy images and graphic representations show the stationary phase support morphology.

Stationary phase morphology

In addition to different column length, all three columns have a unique stationary phase support morphology. The 50 cm µPAC Neo low-load column contains non-porous C18 modified silicon pillars that restricts use to sample loads of 0.1–10 ng of digested protein material. Higher sample loads will not damage the column but decreased separation performance will be observed because the stationary phase will be overloaded. To increase loadability towards more conventional sample amounts (50–500 ng of digested protein material), a complementary 50 cm column with superficially porous silicon pillars is offered. Through electrochemical anodization, a 300 nm thick mesoporous layer is generated within the outer shell of the pillar. Hence, the interaction surface is increased by a factor of approximately 20. With the increased channel cross-section of the 110 cm column, sample loadability is further increased (by a factor 2 compared to the 50 cm column), resulting in a column that can be used for higher sample loads (500–2000 ng) and long gradients. In contrast to conventional reversed-phase nanoLC columns, the interaction surface of all three µPAC Neo columns is significantly lower. This aspect has some limitations, but also creates opportunities such as a reduction in the volume required to fully equilibrate the hydrophobic interaction surface and decreased sample carry-over. Full equilibration of the stationary phase can be achieved with 1.5-column volumes.

Column performance

To assess the chromatographic performance of the µPAC Neo columns, digested protein lysate standards were separated, and peak width distribution was determined based on all peptides identified in a conventional data-dependent 'bottom-up' proteomic experiment. By using all identified peptides rather than a selection of peptides distributed over the elution window, a comprehensive characterization of the chromatographic performance can be obtained. When plotting peak width distributions for different solvent gradients and column lengths, the effect of column length on chromatographic performance becomes apparent (Figure 4). Up to gradient lengths of 60 min, a similar performance was observed for the 50- and 110-cm long columns (median FWHM values of 4 to 5 s). Using a 50-cm long column, however, is more favorable in this range as the shorter column void and equilibration times have a reduced impact on separation productivity. For 90 min gradients and longer, improved chromatography was observed for the 110 cm column; with the greatest difference observed for the longest gradient assessed (240 min, 8.3 s FWHM versus 9.4 s FWHM). Using the peak capacity calculation in Equation 1, peak capacity was used to compare chromatographic performance and the relationship of identified features to the obtained performance was assessed. In line with the observations that the 110 cm long column produces sharper peaks from 90 min gradient lengths and longer, higher peak capacities were obtained with a maximum peak capacity of approximately 1,750 for a 240 min separation gradient.

Equation 1

$$C_p = 1 + \frac{T_G}{\frac{1}{n}\sum_1^n W_p}$$

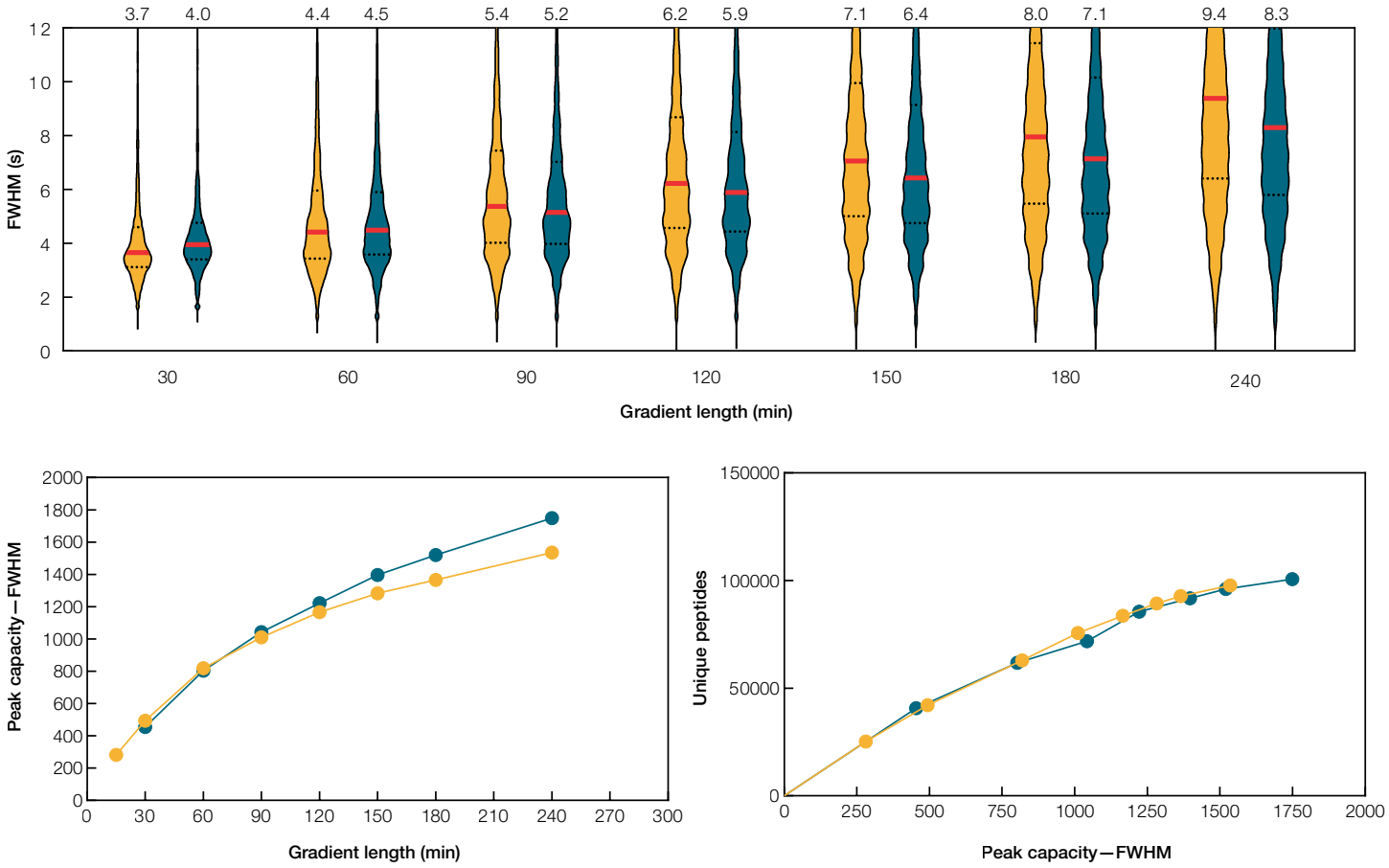


Figure 4. Full-width-at-half-maximum (FWHM) peak width distribution obtained for the separation of a complex HeLa tryptic digest. FWHM values of all identified peptides (n = 40,000 to 105,000) are plotted. Distribution is compared for the 50 (yellow) and 110 cm (teal) µPAC Neo columns. Peak capacity is calculated according to Equation 1 and plotted as a function of gradient length (bottom left). The number of unique peptides identified from 2,000 ng HeLa digest plotted as a function of peak capacity (bottom right).

Proteome coverage

The 50 cm µPAC Neo low-load column gave the best results for sample loads of 1 to 10 ng. On average (n = 3), approximately 2,400 protein groups (1% FDR) were identified from as little as 1 ng of HeLa digest (Figure 5). As the sample load was increased, the number of peptide and protein IDs steadily increased up to 10 ng of material. Further increases in sample load did not result in a significant increase in identifications because of stationary phase overloading and corresponding peak broadening. This was confirmed when comparing the relative gain in peptide IDs that was achieved compared to the superficially porous version of the column (50 cm µPAC Neo column, Figure 6). The increase in proteome coverage was the highest (41%) for the shortest gradient assessed (15 min) and the lowest sample amount loaded (1 ng). Differences became smaller with increasing gradient length and sample load, which is attributed to the non-porous nature of the column.

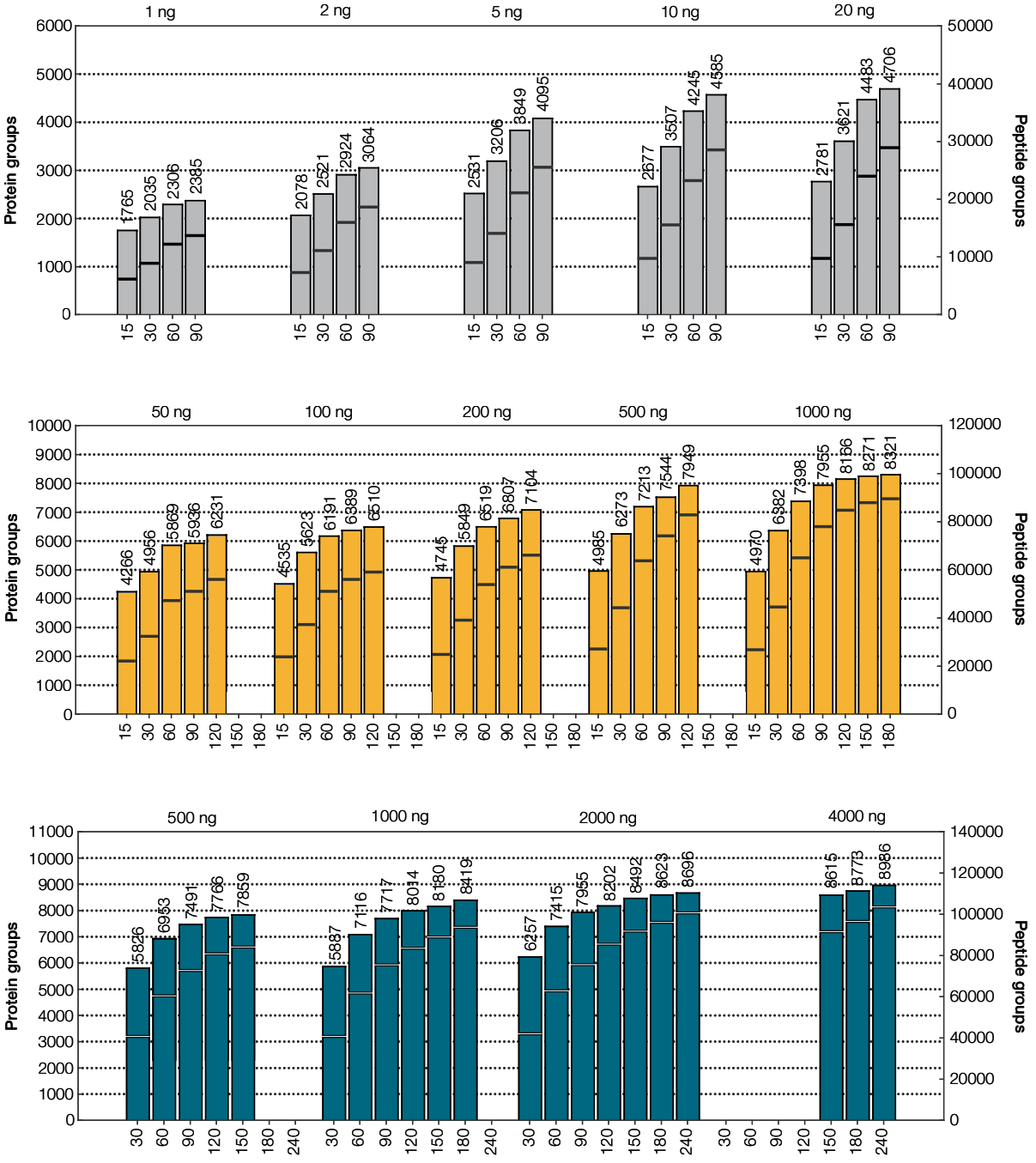


Figure 5. The number of peptides and proteins that were confidently identified on the 50 cm µPAC Neo low-load column (grey), 50 cm µPAC Neo column (yellow), and 110 cm µPAC Neo column (teal).

Using the superficially porous 50 cm µPAC Neo HPLC column, deep proteome coverage for sample loads of between 50 and 1,000 ng of protein digest was obtained. From sample loads of 200 ng, increased peak broadening was observed for peptides of high abundance; however, this had minimal effect on the median peak width and significant increases in proteome coverage were obtained for sample loads up to 1,000 ng. Comparing this data to the 110 cm column, increased proteome depth was obtained for all sample loads assessed up to a gradient length of 120 min. A clear trend toward higher gain from lower sample loads was observed and agrees with expectations (based on chromatographic performance, column length, and loadability). A relative increase in peptide IDs of up to 49% was obtained for the lowest sample amount (100 ng) that was injected on both columns. These results clearly demonstrate the versatile operating range of the 50 cm µPAC Neo column where the potential of performing 'deep dive' 'single shot' analyses (over 8,000 protein group IDs for a 120 min gradient) can be combined with relatively short high throughput analyses (close to 5,000 protein group IDs for 15 min gradients). which makes this column a true all-rounder for proteomic research.

Further increases in proteome coverage were obtained with the 110 cm column with high sample amounts and long gradient times. Consistent identification of approximately 9,000 protein group IDs (n = 3, no MBR, 1% FDR) was achieved. A comparison of the proteome coverage obtained with the 50 cm long column revealed that the effect of the increased separation is only beneficial for long gradient separation times (gradient ≥120 min) and high sample amounts loaded (≥1,000 ng). The increased loadability, however, is also useful when analysing samples with a high dynamic range as sharper elution of the high abundance species will be achieved.

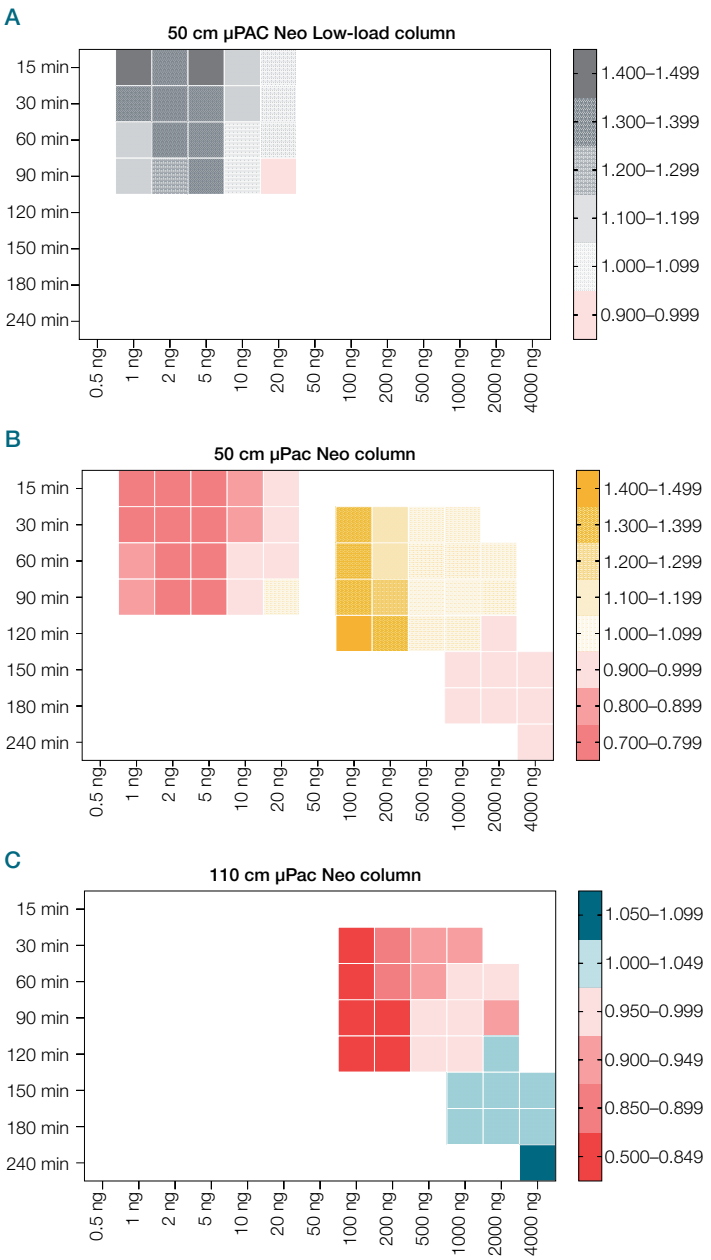


Figure 6. The relative gain in peptide identifications comparing (A) the 50 cm µPAC Neo low-load column with the 50 cm µPAC Neo column, (B) the 50 cm µPAC Neo column with the 110 cm µPAC Neo column and (C) the 110 cm µPAC Neo column with the 50 cm µPAC Neo column.

Reproducibility

As LC-MS-based proteomics is progressively moving towards clinical and translational research and the analysis of large sample cohorts, system robustness and data reproducibility are key factors in the experimental design. Due to the microfabricated nature, µPAC based columns have the inherent potential to reduce inter-column variability. Column-to-column variability was assessed on twelve 50 cm µPAC Neo columns from three different production batches. With a short gradient that was defined to yield maximal sample throughput (20 min separation, peptide elution after 2 min), consistent identification of over 5,000 protein groups were obtained (1.8% RSD at the protein group level, 2.9% RSD at the peptide group level). This method uses flow rate ramping (750 to 250 nL/min) in the first part of the gradient to promote early elution of the peptides and enables operation with a reduced overhead time. Extending the gradient to 67 min and using a constant flow rate of 250 nL/min (80 min total run time), an average of approximately 7,000 proteins were consistently identified on all columns (again 1.8% RSD at the protein group level, 3.6% RSD at the peptide group level), thus highlighting the excellent column-to-column reproducibility that can be achieved.

Longevity

In an ideal world, LC columns would not be consumables, and column replacement would only be due when alternative separation selectivity or column dimensions are required. Unfortunately, reality shows that nanoLC columns often lose separation efficiency or become blocked with intensive use or improper sample preparation. This can have a serious impact on instrument productivity and can result in the loss of precious sample material or require the reanalysis of samples. To investigate column robustness and longevity, a set of columns were aged with up to 1,000 injections of 1,000 ng of HeLa digest sample over a period of 2 months, and these data were then included in an evaluation of reproducibility (Figure 7, column 11 and 12, red bars). In comparison of the proteome coverage with a set of columns that had a limited injection history (<10 injections), no significant differences were observed for the 67 min gradient analyses (student t-test, p-value protein IDs 0.81, p-value peptide IDs 0.99) and only a very mild effect was observed for the short 20 min gradient analyses (student t-test, p-value protein IDs 0.03, p-value peptide IDs 0.98). To monitor column performance throughout the longevity evaluation, the separation of a single protein digest was performed at regular intervals (QC analysis after every 100th HeLa injection) on a Thermo Scientific™ UltiMate™ 3000 RSLCnano system equipped with a 3 nL UV flow cell. As aging and performance assessments were analyzed on different nanoLC systems, this evaluation not only incorporated inherent variation but also potential damage incurred through multiple column connection and disconnection. Throughout this period of aging, retention time variation was <2% RSD for all peptides evaluated and no effects on chromatographic performance (FWHM) nor column back pressure were observed.

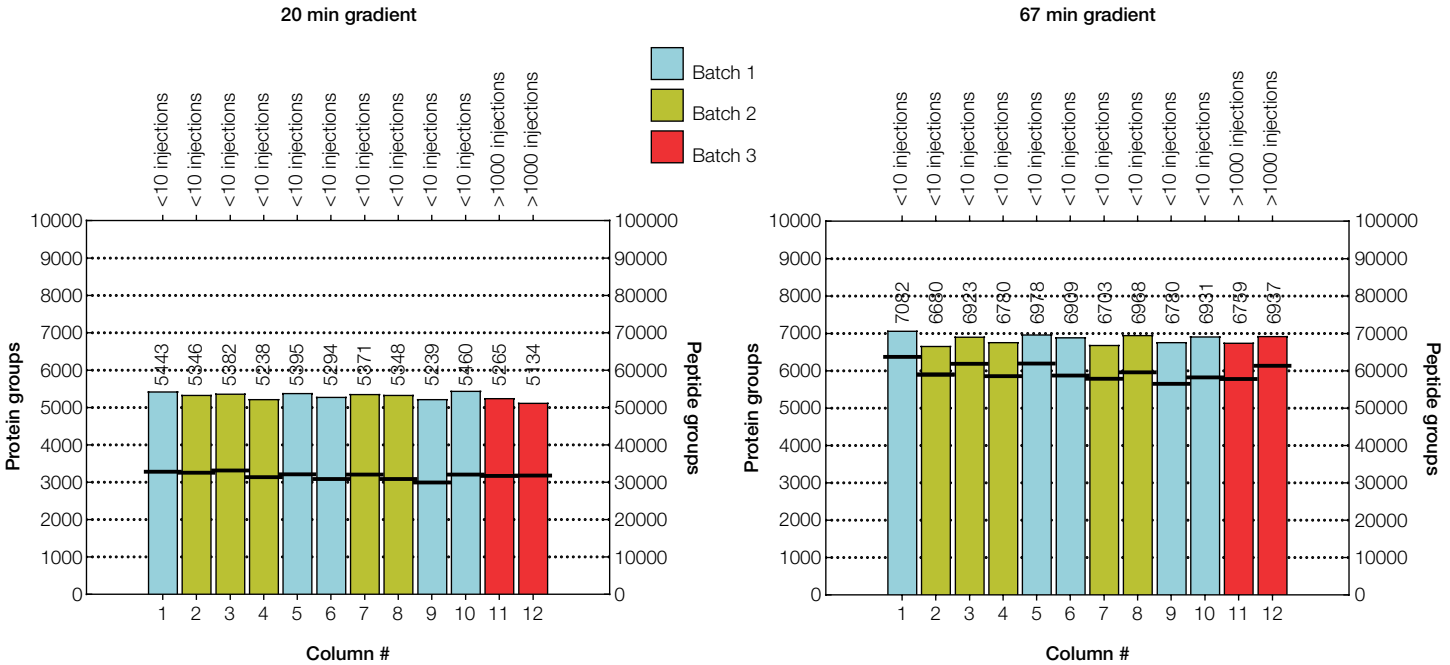


Figure 7. The number of peptide and protein groups identified on twelve 50 cm µPAC Neo columns from three different production batches. (A) 20 min gradient method (B) 67 min gradient method.

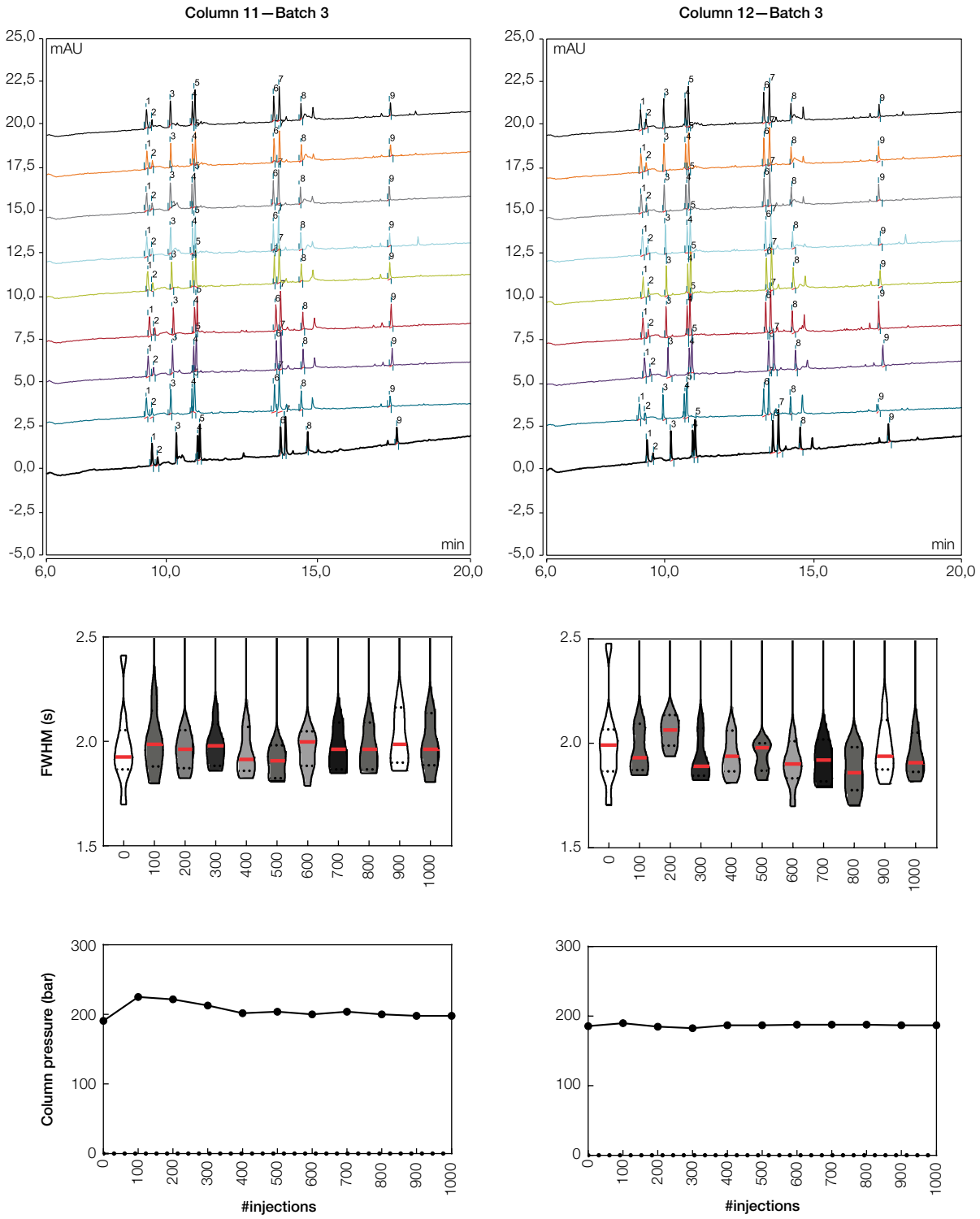


Figure 8. UV chromatograms obtained for intermediate quality control analyses in the longevity experiment. A tryptic digest of Cytochrome C digest was analysed after every 100th HeLa digest injection (top). FWHM values of 9 selected peptides are used to monitor chromatographic performance over time (middle). The pressure recorded at the beginning of each QC analysis is also plotted (bottom).

Conclusion

Using a Vanquish Neo UHPLC system coupled to an Orbitrap Exploris 240 mass spectrometer, the performance and the proteomic coverage that can be obtained with all three µPAC Neo HPLC columns was evaluated. For analyses that require maximum sensitivity for low sample amounts, the non-porous 50 cm µPAC Neo low-load column is recommended. Superior performance can be expected for sample loads up to 10 ng of protein digest and for analyses below 2 hours. High to medium throughput analyses with more conventional nanoLC peptide amounts will benefit from the increase in the interaction surface provided by the superficially porous 50 cm µPAC Neo column. The versatility of the 50 cm µPAC Neo column enables both high throughput and 'deep dive' proteomic profiling on a single column thus ensuring that this column is a true all-rounder for proteomic research. And finally, even higher sample amounts and comprehensive 'single-shot' experiments will benefit from the unrivalled peak capacity of the superficially porous 110 cm µPAC Neo column. Integration of the nanoViper Fingertight fittings enables easy, tool-free, and reliable nanoLC column installation. The monolithic nature of the microfabricated pillar beds enables fast flow rate ramping, requires significantly less equilibration time, and is much less prone to the effects of sample carry-over. The results presented here show excellent stability and column-to-column reproducibility, even after 1,000 complex digest injections, thus providing a foolproof nanoLC solution for consistent and continuous data generation.

References

1. Mazzeo, J. R., Neue, U. D., Kele, M., Plumb, R. S. & Corp, W. A new separation technique takes advantage of sub-2-µm HPLC. *J Am Chem Soc* 460–467 (2005).
2. Lopez-Ferrer, D. *et al.* Pushing the Limits of Bottom-Up Proteomics with State-Of-The-Art Capillary UHPLC and Orbitrap Mass Spectrometry for Reproducible Quantitation of Proteomes. *Thermo Fisher Scientific Application Note AN639* (2016).
3. de Malsche, W., op de Beeck, J., de Bruyne, S., Gardeniers, H. & Desmet, G. Realization of 1 × 10 6 theoretical plates in liquid chromatography using very long pillar array columns. *Anal Chem* **84**, 1214–1219 (2012).
4. Doblmann, J. *et al.* ApQuant: Accurate Label-Free Quantification by Quality Filtering. *J Proteome Res* **18**, 535–541 (2019).
5. Gessulat, S. *et al.* Prosit: proteome-wide prediction of peptide tandem mass spectra by deep learning. *Nat Methods* **16**, 509–518 (2019).

Learn more at **thermofisher.com/lowflowHPLCcolumns**