AURORA® 5 SERIES

TECHNICAL NOTE

Aurora® Elite™ XT 15 × 75 C18

Deeper coverage.
Sustained at speed.

Peak properties, identification rates, quantitative precision and column lifetime for the Aurora® Elite™ XT 15 × 75 column on a Thermo Scientific Orbitrap™ Astral™ Zoom, from 40 to 120 samples per day and across more than 1,350 injections.

Series 5 Aurora® Elite™ 15 × 75 XT provides robust separation at high throughput.

> 10,280	> 9,420	1.13 s	1,350+
PROTEINS QUANTIFIED AT 40 SPD	PROTEINS QUANTIFIED AT 120 SPD	MEDIAN FWHM AT 120 SPD, ± 0.05 S	HIGH-PRESSURE INJECTION CYCLES

Tested across a range of sample inputs and throughputs.

This note sets out what the Aurora® Elite™ XT 15 cm × 75 µm column delivers across a range of sample inputs and gradient throughputs, and shows it holding that performance across more than 1,350 injections.

To benchmark the performance of the IonOpticks Aurora® Elite™ 15 cm × 75 µm column, we separated 20, 50 and 200 ng of HeLa digest standard at 40, 80 and 120 SPD. Peak parameters, identification rates and quantitative precision were evaluated using optimised gradients.

To evaluate column longevity, we monitored performance metrics and pressure stability over > 1,350 high-pressure injection cycles by periodically analysing a 50 ng HeLa standard at 120 SPD (6.4-minute gradient).

IN THIS NOTE

Identifications and precision	Fig. 1
Peak width and signal	Fig. 2–3
Coverage and precision over 1,350+ runs	Fig. 4–6
Pressure and chromatographic stability	Fig. 7–8
Methods and parameters	Tables 1–6

INSTRUMENTATION USED



MASS SPECTROMETER
Thermo Scientific Orbitrap™ Astral™ Zoom with EASY-Spray™ source



UHPLC
Thermo Scientific Vanquish™ Neo pump and autosampler



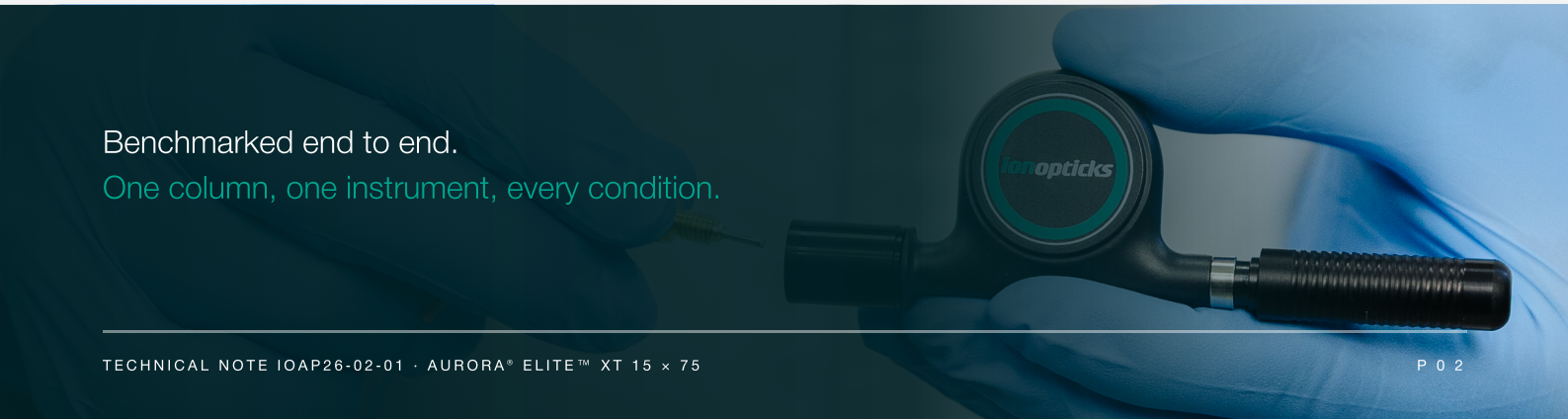
DATA PROCESSING
DIA-NN Enterprise 2.6.1
© Aptila Biotech GmbH



COLUMN
IonOpticks Aurora® Elite™ XT 15 × 75 C18 UHPLC column



COLUMN HEATING
IonOpticks HeatSync™ system set at 50 °C



Benchmarked end to end.
One column, one instrument, every condition.

Deep coverage, with precision to match.

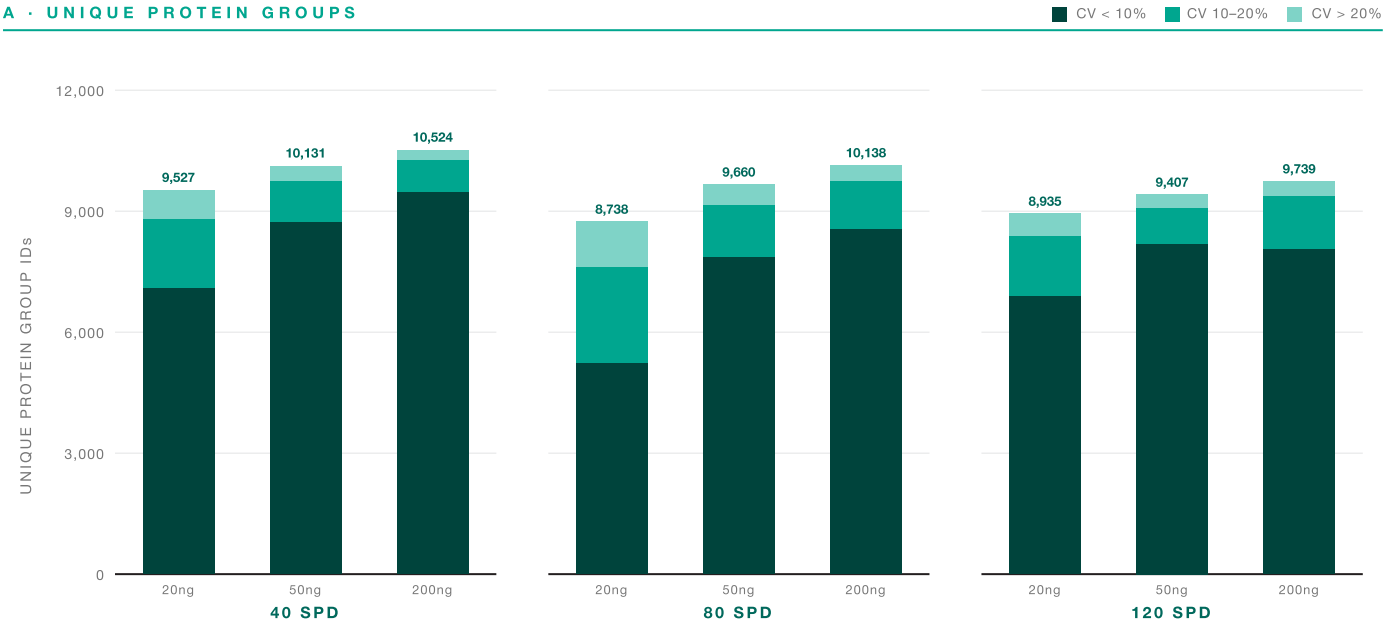
10,280
PROTEINS QUANTIFIED* AT 40 SPD

9,420
PROTEINS QUANTIFIED* AT 120 SPD

Coverage only counts if it quantifies reproducibly. From 200 ng at 40 SPD the column quantifies 10,280 protein groups; at 120 SPD, where each run lasts 6.4 minutes, 9,420 are still quantified. Peptides and protein groups were considered as quantified if their CV across five replicates was <20%.

*Quantified proteins are those with CV < 20%.

A · UNIQUE PROTEIN GROUPS



B · UNIQUE PEPTIDES

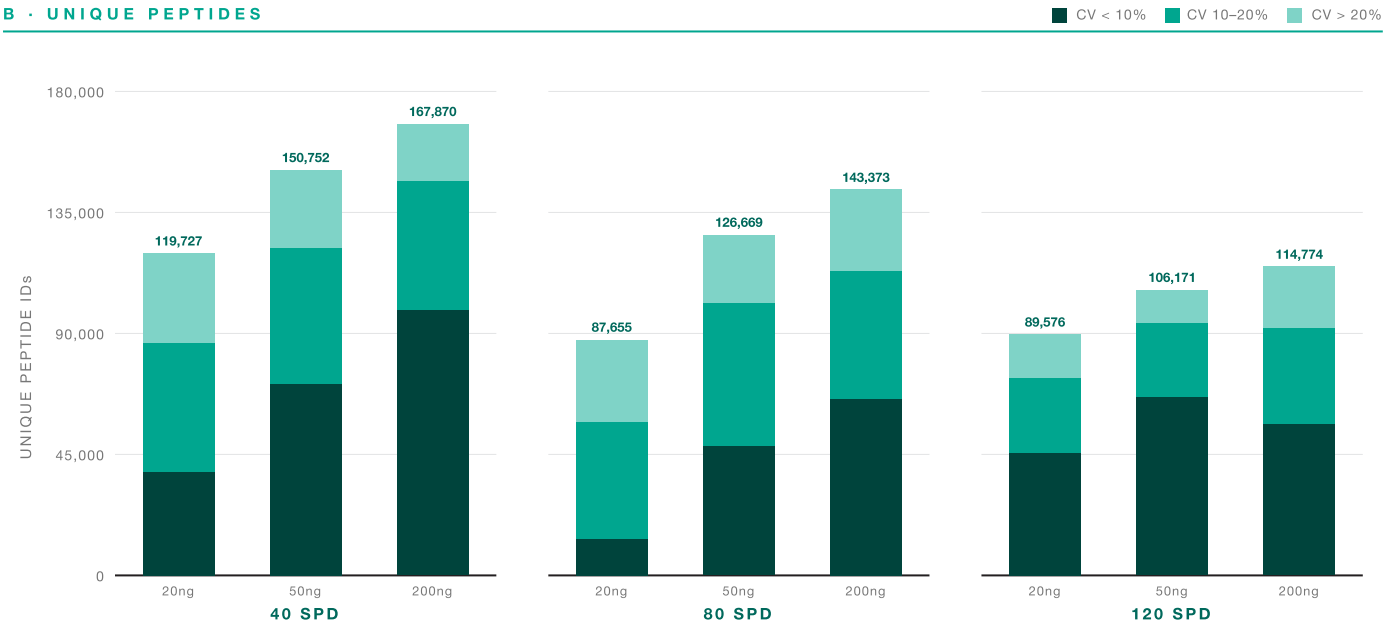


Figure 1: The number of protein groups (A) and unique peptides (B) identified for runs from differing inputs of either a 20 ng, 50 ng or 200 ng HeLa digest standard (n = 5), separated using Aurora® Elite™ 15 cm × 75 µm. The identifications are grouped by gradient throughput and CV: < 10% (dark), 10–20% (mid) and > 20% (light).

MORE PEPTIDES PER RUN

167,870 unique peptides from 200 ng at 40 SPD.

QUANTIFIED WITH CONFIDENCE

10,280 protein groups at CV < 20% from 200 ng at 40 SPD.

HOLDS AT 120 SPD

9,420 protein groups still quantified in a 6.4-minute gradient.

Narrow peaks at every throughput.

< 3 s FWHM AT 40 SPD

< 1.2 s FWHM AT 120 SPD

Peak width sets the ceiling on everything downstream. The Aurora® Elite™ 15 cm × 75 µm column holds narrow peak widths across a range of throughputs, and narrow peaks mean increased signal intensity.

PRECURSOR PEAK WIDTH DISTRIBUTION

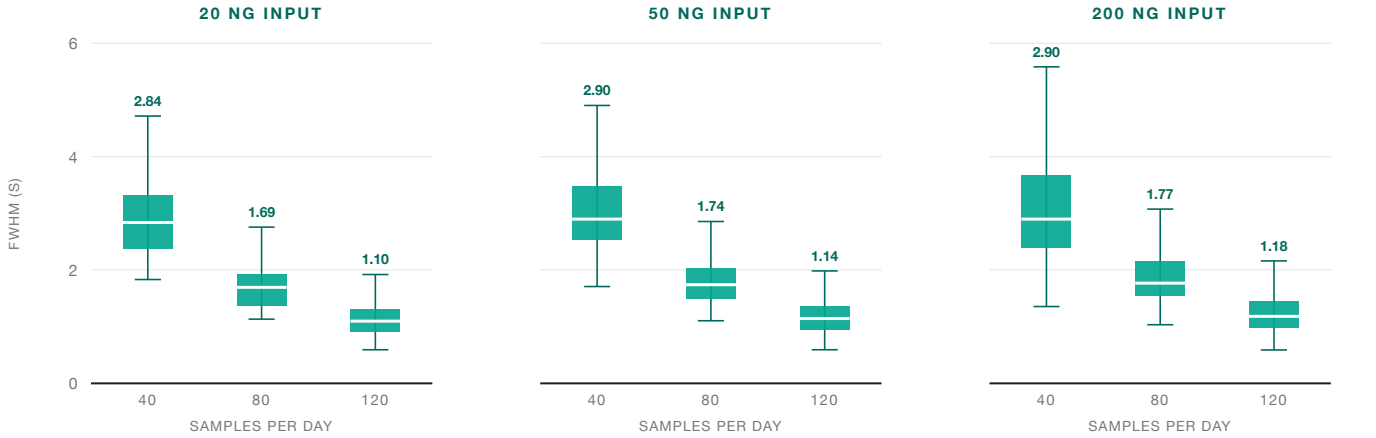


Figure 2: 20 ng, 50 ng or 200 ng of HeLa digest standard was separated on an Aurora® Elite™ 15 cm × 75 µm column using a 40 SPD, 80 SPD or 120 SPD gradient (n = 5). Box plot of precursor peak width distributions, with the median marked on each box.

AVERAGED BASE PEAK CHROMATOGRAM · 80 SPD · 50 NG

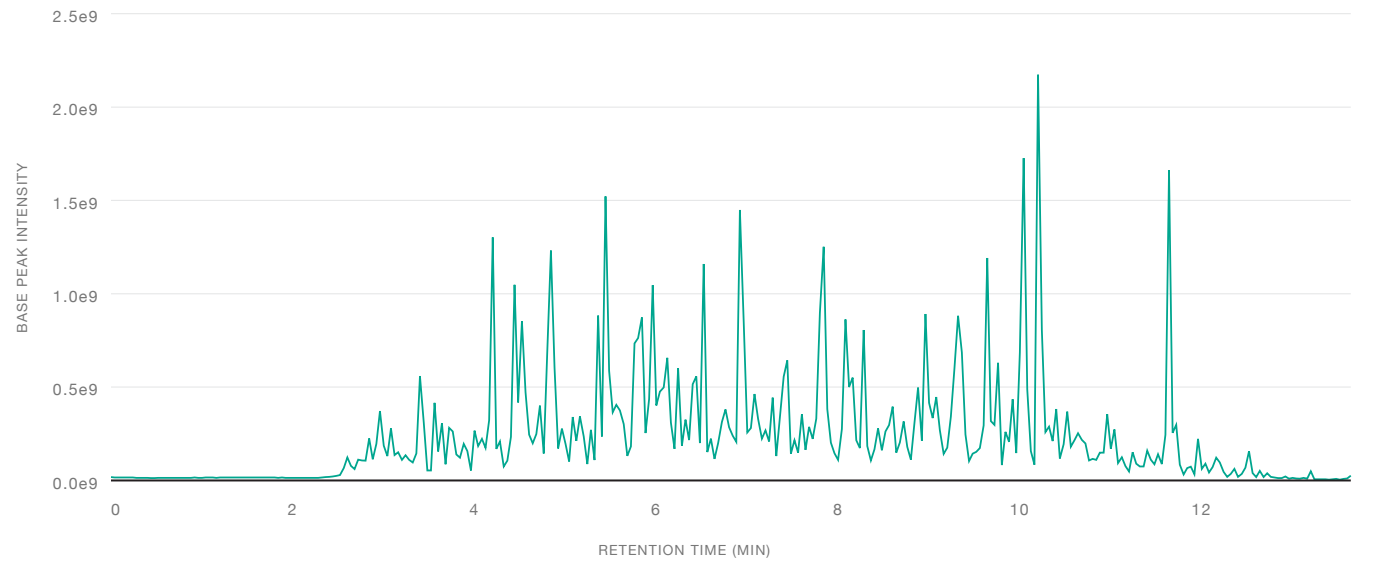


Figure 3: Averaged base peak chromatogram (n = 5) of a 50 ng HeLa digest standard separated at 80 SPD throughput using the IonOpticks Aurora® Elite™ 15 cm × 75 µm column.

SHARP AT EVERY LOAD

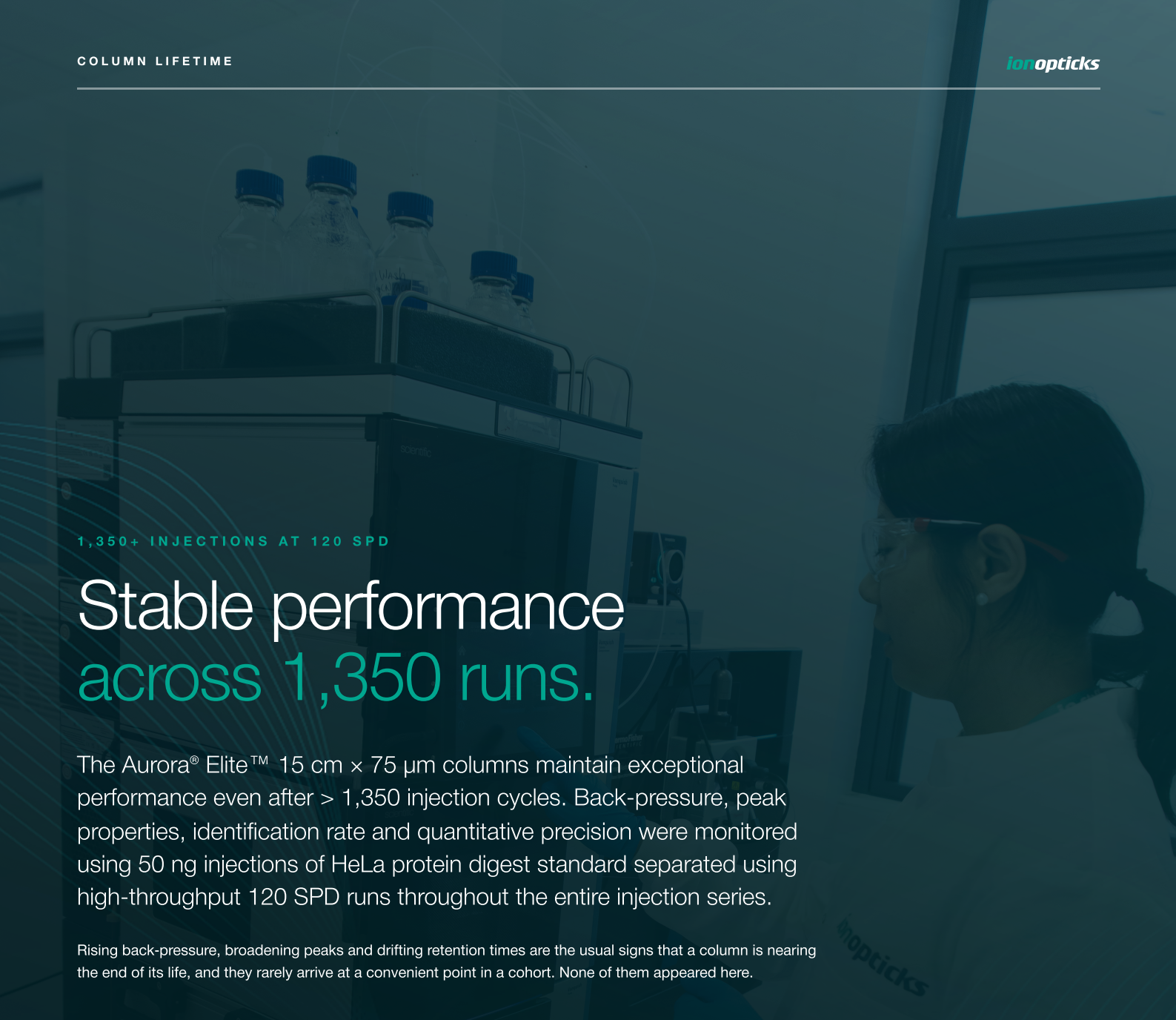
Median FWHM within 0.08 s across 20 to 200 ng.

SHARPER WITH THROUGHPUT

Median FWHM falls from 2.84 s at 40 SPD to 1.10 s at 120 SPD.

MORE SIGNAL PER PEAK

Sharper peaks concentrate the same sample into a taller signal.



1,350+ INJECTIONS AT 120 SPD

Stable performance across 1,350 runs.

The Aurora® Elite™ 15 cm × 75 µm columns maintain exceptional performance even after > 1,350 injection cycles. Back-pressure, peak properties, identification rate and quantitative precision were monitored using 50 ng injections of HeLa protein digest standard separated using high-throughput 120 SPD runs throughout the entire injection series.

Rising back-pressure, broadening peaks and drifting retention times are the usual signs that a column is nearing the end of its life, and they rarely arrive at a convenient point in a cohort. None of them appeared here.

> 9,600
PROTEIN GROUPS PER RUN

9,036
PROTEIN GROUPS SHARED ACROSS ALL RUNS

r > 0.99
LOG_2 INTENSITY CORRELATION

0.58%
BACK-PRESSURE COEFFICIENT OF VARIATION

Coverage that does not drift across a cohort.

9,036
PROTEIN GROUPS IN EVERY SINGLE RUN

1.13 s
MEDIAN FWHM ± 0.05 S

The protein identification rate remained high across the dataset, consistently exceeding 9,600 proteins per run. Of the identified protein groups, 9,036 were shared across all runs, with a Log_2-intensity Pearson correlation coefficient of > 0.99. The peak capacity of the column also remained stable: eluting peaks stayed symmetrical and the peak width at half maximum height held at around 1.13 ± 0.05 s.

UNIQUE PROTEIN GROUPS PER RUN

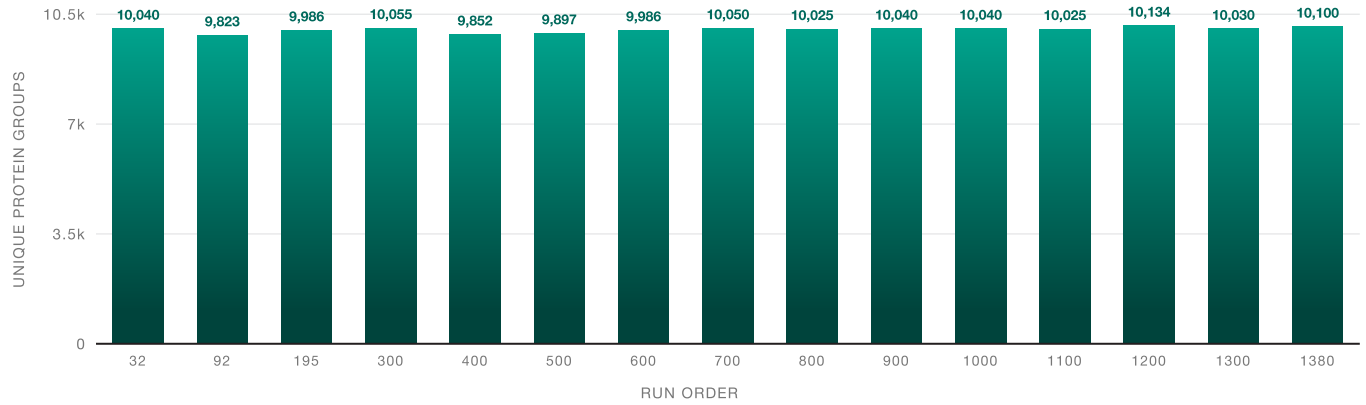
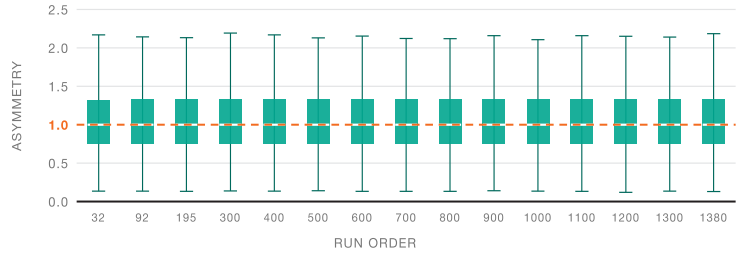


Figure 4: Number of unique protein groups identified from runs throughout a > 1,350 injection series. Data points represent 50 ng HeLa digest standard injections separated at 120 SPD throughput (6.4-minute gradient).

A · PEAK ASYMMETRY FACTOR



B · FULL WIDTH AT HALF MAXIMUM

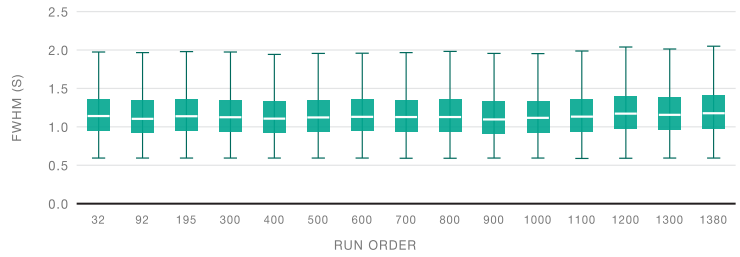


Figure 5: Peak shape parameter distributions across injections of a 50 ng HeLa digest standard separated with the Aurora® Elite™ 15 cm × 75 µm column at 120 SPD throughput. (A) Individual box plots tracking the peak asymmetry factor for each monitored QC run throughout a > 1,350 injection series. (B) Individual box plots tracking the full width at half maximum (FWHM).

PEARSON r, LOG_2 INTENSITY

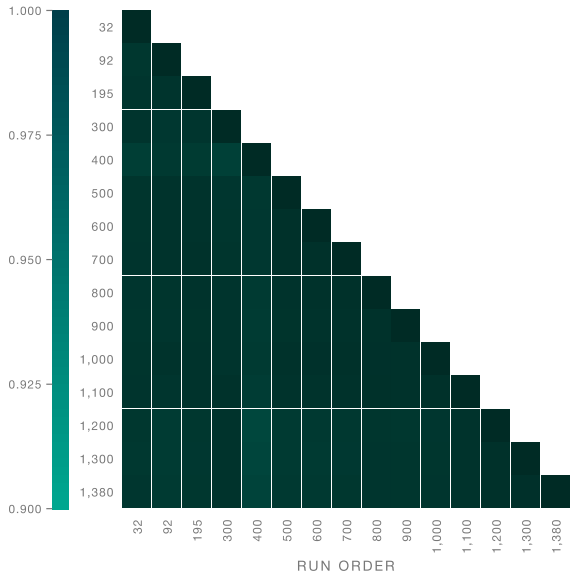


Figure 6: Pearson correlation coefficient of Log_2 protein intensities for the 9,036 protein groups identified across the HeLa standard runs over 1,350 injections.

DEPTH HELD

More than 9,600 protein groups per run, start to finish.

THE SAME PROTEINS

9,036 groups in every run, correlating above r = 0.99.

PEAK SHAPE INTACT

Asymmetry at 1.00 and FWHM at 1.13 ± 0.05 s throughout.

No creep in pressure, no drift in profile.

< 5 bar
CHANGE IN PRESSURE OVER 1,350 INJECTIONS

0.58%
BACK-PRESSURE COEFFICIENT OF VARIATION

The maximum back-pressure recorded during the gradient throughout the entire series remained stable, with a coefficient of variation of 0.58%. Chromatographic profile stability was maintained uniformly across the entire gradient window, with stable signal intensities and retention times throughout the > 1,350 injection series.

MAXIMUM BACK-PRESSURE PER RUN

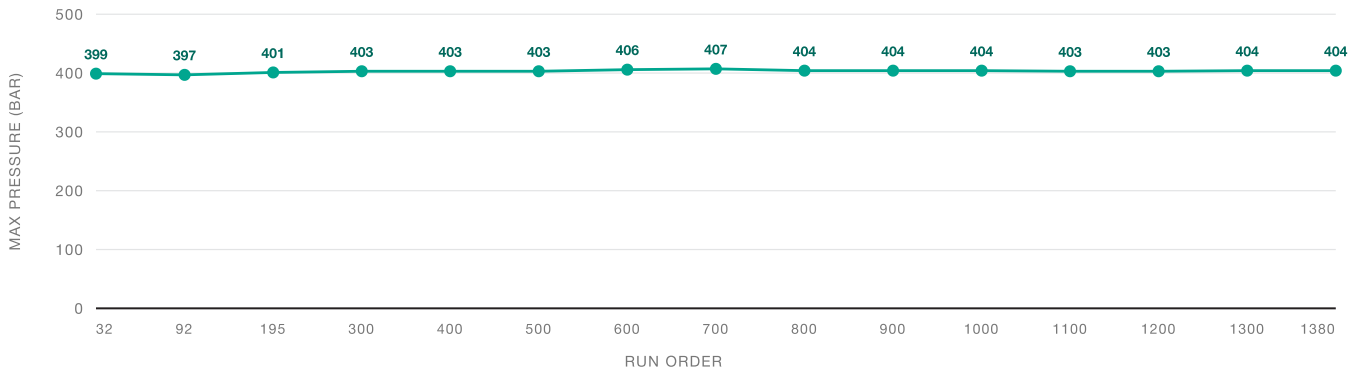
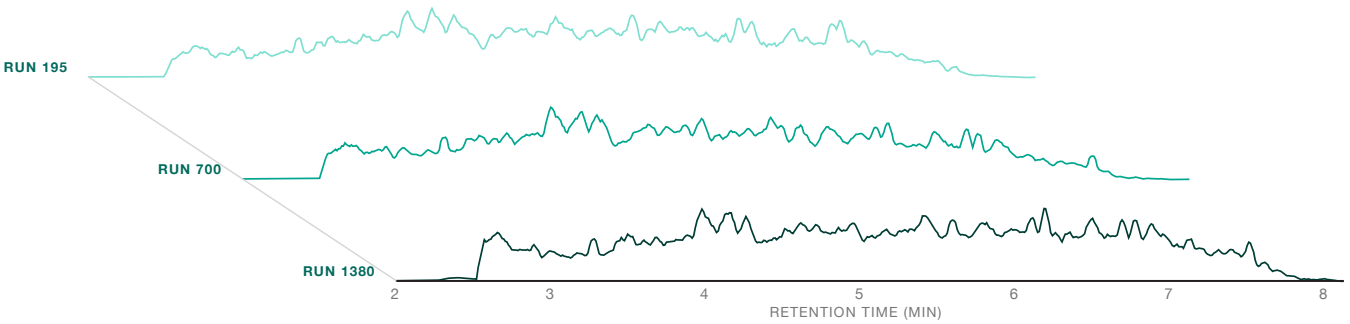


Figure 7: Maximum back-pressure recorded during each of the 120 SPD gradients throughout a > 1,350 injection series.

A · TIC CHROMATOGRAM CASCADE



B · EXTRACTED ION CHROMATOGRAMS

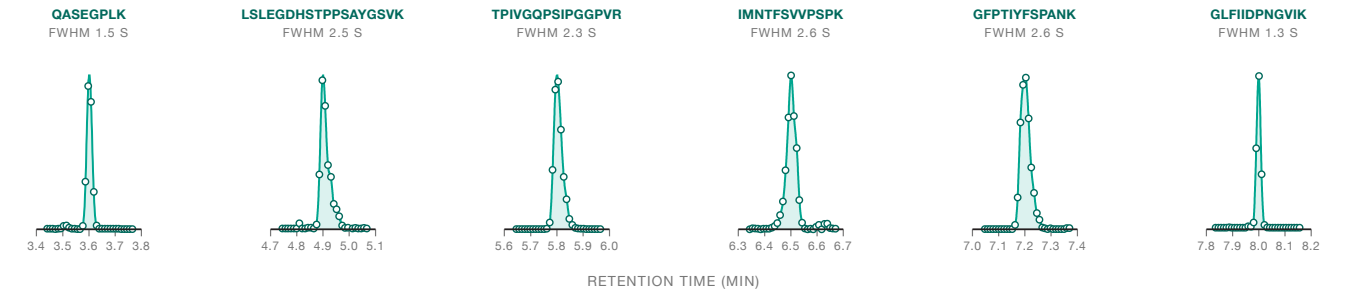


Figure 8: (A) Total ion chromatograms for runs 195, 700 and 1,380, cascaded and offset for clarity. (B) Extracted ion chromatograms of six representative precursor peaks, each labelled with its peak width at half maximum.

STABLE SPRAY

Continuous TIC traces from the first run to the last.

PEAK SHAPE HELD

Each precursor keeps its width and retention time across the series.

PEAKS FULLY RESOLVED

Every extracted peak is defined by multiple points across its width.

Methods and parameters.

Peptides were separated on an Aurora® Elite™ 15 × 75 column connected to the Thermo Scientific™ Vanquish™ Neo UHPLC system using a 20 µm ID nanoViper transfer line. The UHPLC was configured for a direct injection workflow. The Orbitrap™ Astral™ Zoom MS was operated in “Peptide” application mode with acquisition parameters optimised to maximise the number of identified precursors for each gradient length.

TABLE 1 · UHPLC SETUP

Mobile phase A	0.1% formic acid (FA) in water
Mobile phase B	0.1% FA in 99.99% acetonitrile (ACN)
Gradient flow rate	0.4 µL/min
Column	Aurora® Elite™ XT 15 cm × 75 µm
Column temperature	50 °C
Autosampler temperature	7 °C
Injection wash solvents	Strong: 0.1% FA in 80% ACN Weak: 0.1% FA in water
Needle wash	Enabled, after-draw
Loading volume	Automatic
Fast loading and equilibration	Pressure control

TABLE 2 · COLUMN SETTINGS

Inner diameter	75 µm
Length	15.0 cm
Void volume	0.444 µL
Maximum pressure	1450 bar
Maximum flow	5.0 µL/min
Maximum temperature	60 °C
Max. pressure change up	1000 bar/min
Max. pressure change down	1000 bar/min

Consumables: Fisher Scientific™ LC-MS Optima formic acid (A117-50), Optima™ LC-MS grade water (10505904), acetonitrile (A955-1) and isopropanol (A461-212); Thermo Scientific™ Pierce™ HeLa protein digest standard (88328).

TABLES 3–5 · GRADIENT PARAMETERS

Time and duration in minutes; flow rate in µL/min. Gradients were optimised for each throughput.

Table 3 · 40 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	0.400	1.0
0.050	0.050	0.400	3.0
25.500	25.450	0.400	29.0
25.800	0.300	0.400	32.0
26.000	0.200	0.400	85.0
28.000	2.000	0.400	85.0

Table 4 · 80 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	0.400	1.0
0.050	0.050	0.400	4.0
11.450	11.400	0.400	32.0
11.650	0.200	0.400	40.0
11.750	0.100	0.400	85.0
12.950	1.200	0.400	85.0
13.000	0.050	0.400	1.0
14.000	1.000	0.400	1.0

Table 5 · 120 SPD

TIME	DUR.	FLOW	%B
0.000	0.000	0.400	1.0
0.050	0.050	0.400	5.0
6.450	6.400	0.400	33.0
6.500	0.050	2.500	85.0
8.200	1.700	0.400	85.0
8.250	0.050	0.400	1.0

TABLE 6 · MS DATA ACQUISITION PARAMETERS

Source and method

Source voltage	1.6–1.8 kV
Transfer tube temp.	280 °C
Polarity	Positive
Expected LC peak width	3 s
Advanced peak determination	On
Default charge state	2
Lock mass correction	Off

Orbitrap MS1

Resolution	240,000
Enhanced dynamic range	Off
Scan range (m/z)	380–980 (40/80 SPD) 400–800 (120 SPD)
RF lens	45%
Normalised AGC target	500%
Maximum injection time	3 ms
Data type	Profile

Astral MS2

Acquisition mode	Data-independent
Precursor range (m/z)	380–980 (40/80 SPD) 400–800 (120 SPD)
Isolation window	4 m/z
Window overlap	0
Collision energy	25%
Scan range (m/z)	150–2000
Max. injection time	6 / 3 / 2 ms

Maximum injection time given for 40 / 80 / 120 SPD. Window placement optimisation and pre-accumulation on; normalised AGC target 500%; loop control time 0.6 s. Raw data files were processed in DIA-NN Enterprise (version 2.6.1) using a custom spectral library generated via a library-free search of 2–3 hour DIA HeLa runs acquired on an IonOpticks Aurora® Frontier™ column. Raw files were processed in batches grouped by column and gradient length with match-between-runs disabled. Mass tolerances were 4 ppm for MS1 and 10 ppm for MS2. Quantification used Legacy (direct) mode with global normalisation enabled.

SUMMARY

The IonOpticks Aurora® Elite™ 15 cm × 75 µm column delivers superior separation efficiency, durability and quantitative performance, holding sub-1.2 second peak widths and above 9,400 quantified proteins at 120 samples per day.

Backed by an extended column lifetime study at 120 SPD, the column maintains this performance over 1,350 high-pressure injection cycles, consistently exceeding an identification rate of 9,600 proteins per run. The Aurora® Elite™ architecture successfully eliminates the trade-off between throughput, sensitivity and column lifespan.

IOAP26-02-01 · **FOR RESEARCH USE ONLY.** Not for use in diagnostic procedures.

‘IonOpticks’ and all represented trademarks remain the property of Ion Opticks Research Pty Ltd, and its related companies, unless otherwise specified. ‘Orbitrap’, ‘Astral’ and ‘Vanquish Neo’ are all trademarks of Thermo Fisher Scientific.

ionopticks

© 2026 IonOpticks Pty Ltd. All Rights Reserved.

ABN 99 621 674 459 | **www.ionopticks.com**