AURORA® SERIES 5

APPLICATION NOTE

Aurora® Rapid™ XT 8 × 75 C18

CoreJet™ integrated emitters maintain performance and consistent back-pressure across 1,200 laser microdissection sample injections.

1,200 LMD tissue injections made possible by the NEW CoreJet™ integrated emitter, redesigned for robustness.

AUTHORS

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KEYWORDS

Aurora Series 5 · Aurora Rapid XT 8 cm × 75 µm C18 · CoreJet integrated emitter · Laser microdissection (LMD) · Tissue proteomics · Spatial proteomics · Low-input proteomics · HeLa QC standard · Column robustness · Column lifetime · Back-pressure stability · Spray stability · Peak width (FWHM) · Retention time stability · Trap-and-elute · Nano-flow UHPLC · Thermo Scientific Vanquish Neo · Orbitrap Astral · FAIMS · Data-independent acquisition (DIA) · DIA-NN · High-throughput proteomics

1,200+
LMD TISSUE INJECTIONS ON A SINGLE COLUMN

~90 bar
BACK-PRESSURE ± 5 BAR, FIRST RUN TO LAST

1.6-1.7 s
MEDIAN FWHM AT EVERY QC CHECKPOINT

> 0.98
AVERAGE PEARSON CORRELATION

Large spatial cohorts need a column that keeps running.

Spatial proteomics workflows such as laser microdissection (LMD) now generate cohorts of hundreds to thousands of samples. These samples are difficult to run: protein input is low, clean-up is minimal, and residual contaminants such as salts, detergents and tissue debris gradually clog columns and emitters, raising back-pressure and degrading performance. Large LMD studies therefore need a nanoflow LC-MS system that is sensitive and robust enough to run injection after injection.

The Aurora Series® 5 column, featuring new CoreJet™ integrated emitter technology, makes this possible. On a Thermo Scientific Vanquish Neo-Orbitrap Astral system, a single Aurora® Rapid™ XT 8 cm × 75 µm column enabled more than 1,200 LMD tissue injections, delivering stable back-pressure, consistent chromatographic performance and deep proteome coverage from the first injection to the last.

INSTRUMENTATION USED



MASS SPEC

Thermo Scientific Orbitrap Astral with FAIMS



UHPLC

Thermo Scientific Vanquish Neo, trap-and-elute mode



COLUMN

IonOpticks Aurora® Rapid™ XT 8 cm × 75 µm C18 with CoreJet™ integrated emitter



DATA PROCESSING

DIA-NN Enterprise 2.6.1, © Aptila Biotech GmbH

Column back-pressure stability.

More than 1,200 LMD tissue samples were analysed on the Aurora® Rapid™ XT 8 cm × 75 µm column fitted with a CoreJet™ integrated emitter, using an 8.5-minute method. System performance was monitored using a 50 pg HeLa digest injected after every 130 tissue samples, giving 11 QC checkpoints across the column's lifetime. No emitter clogging, overpressure events or emitter replacements occurred across the series. Back-pressure remained at approximately 90 bar (± 5 bar) from the first injection to the last, with no upward drift (Figure 1).

0

CLOGGING OR OVERPRESSURE EVENTS

± 5 bar

BACK-PRESSURE ACROSS THE SERIES

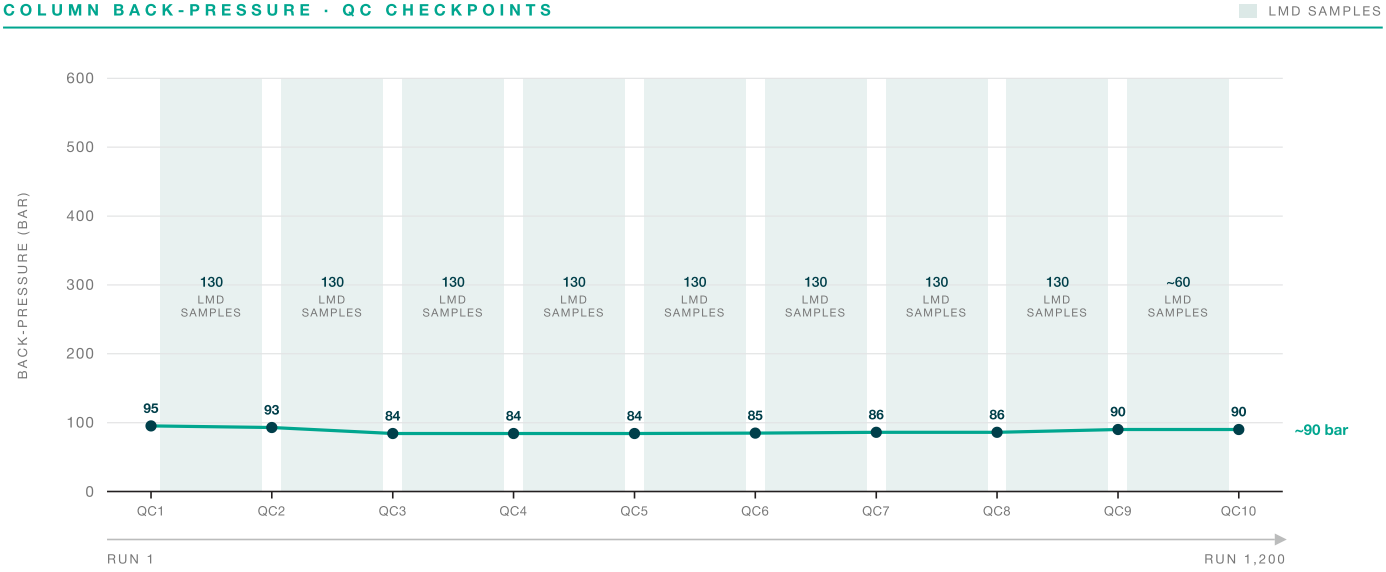


Figure 1. Stable pressure from run 1 through run 1,200 on the Aurora® Rapid™ XT 8 cm × 75 µm column (Thermo Scientific Vanquish Neo-Orbitrap Astral). Back-pressure remained flat across the full run series, with no upward drift observed over the lifetime of the column. Real LMD tissue samples were analysed between each QC injection.

Complex samples, uncompromised depth.

~3,300
PROTEINS FROM 50 PG HELA

~13,600
PEPTIDES FROM 50 PG HELA

11
QC RUNS WITH MATCHED RT

Even after more than 1,200 consecutive tissue injections, proteome coverage was maintained across the series, with an average of ~3,300 protein and ~13,600 peptide identifications from 50 pg HeLa across the 11 QC injections (Figure 2). The gradual dip in identifications over the later checkpoints, followed by the rebound at the final run, tracks the QC standard rather than the column. A fresh HeLa QC sample was introduced for the last QC checkpoint and identifications immediately rose to ~3,800 proteins and ~15,800 peptides.

Retention times were equally stable. Selected precursors eluted at identical times in every QC run (Figure 3).



Figure 2. Protein (A) and peptide (B) identifications from 50 pg HeLa QC injections across > 1,200 injections on the Aurora® Rapid™ XT 8 cm × 75 µm column with CoreJet™ integrated emitter (Thermo Scientific Vanquish Neo-Orbitrap Astral). Each QC followed ~130 LMD tissue samples and 2 blanks. Data analysed with DIA-NN Enterprise 2.6.1 (© Aptila Biotech GmbH).

RETENTION TIME · TOP PRECURSORS ACROSS THE GRADIENT

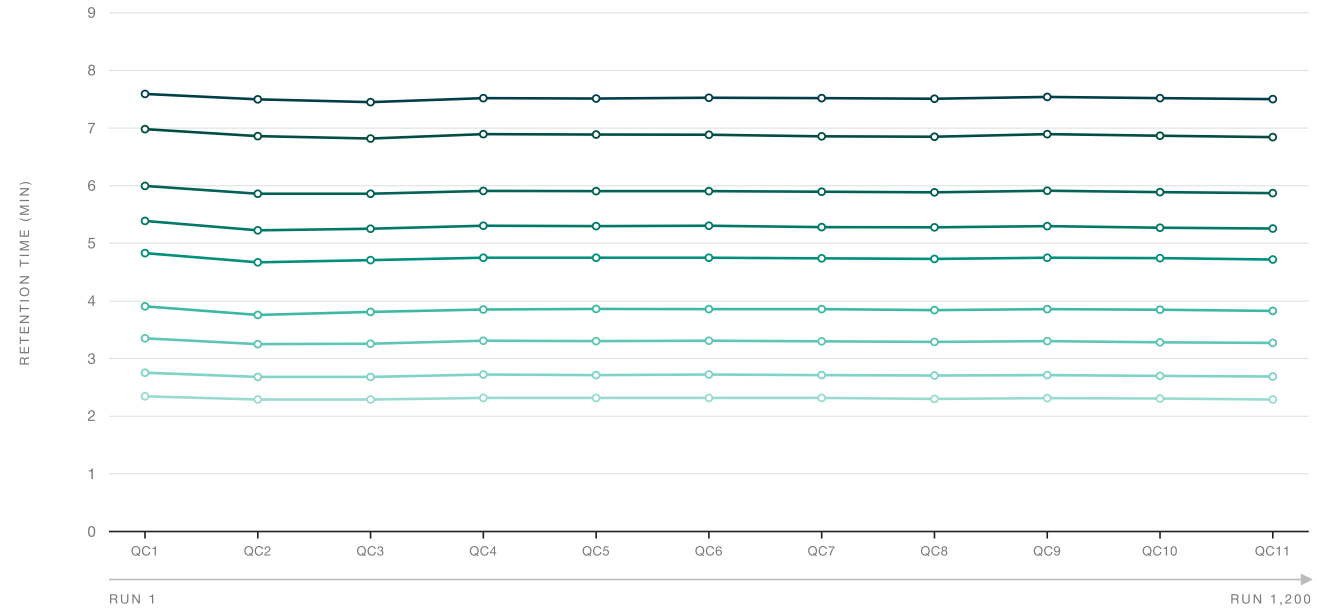


Figure 3. Retention times of selected precursors spanning the gradient across all QC injections, showing consistent elution over the > 1,200 injection series.

Sharp peaks and reproducible quantitation.

1.6-1.7 s
MEDIAN FWHM, EVERY QC RUN

> 0.98
AVERAGE PEARSON CORRELATION

Median peak width (FWHM) held at ~1.6-1.7 s in every QC run, from the first checkpoint to the last (Figure 4). Peaks remained sharp even after 1,200 complex tissue samples had passed through the system.

Quantitative reproducibility was similarly robust, with an average Pearson correlation of > 0.98 for protein intensities across all QC injections (Figure 5).

PEAK WIDTH (FWHM) · QC CHECKPOINTS

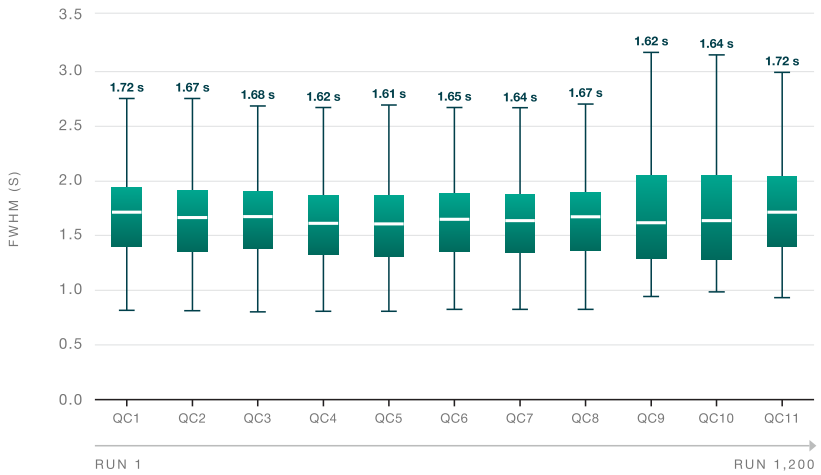


Figure 4. Peak width (FWHM) distributions across 11 QC injections of a 50 pg HeLa Pierce digest standard on the Aurora® Rapid™ XT 8 cm × 75 µm column (Thermo Scientific Vanquish Neo-Orbitrap Astral). Box plots track the full width at half maximum (FWHM, in seconds) for each monitored QC run throughout a > 1,200 injection series. Boxes show the interquartile range, the white line the median and whiskers the range.

PEARSON CORRELATION

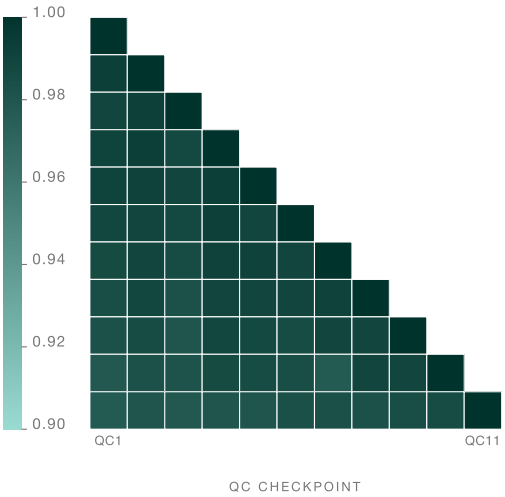


Figure 5. Pearson correlation of quantified protein intensities across all 11 QC injections, throughout a > 1,200 injection series. Real LMD tissue samples were analysed between each QC injection.

METHODS

A Thermo Scientific Vanquish Neo UHPLC was operated in trap-and-elute mode using a Thermo Scientific PepMap Neo trap cartridge paired with a Series 5 Aurora® Rapid™ XT 8 cm × 75 µm column fitted with a CoreJet™ integrated emitter. Mobile phases were 0.1% formic acid in water (A) and 0.1% formic acid in 80% acetonitrile (B). A 5-40% B gradient was run at 0.9 µL/min within the first 1.2 min, then at 0.15 µL/min for separation, followed by a 99% B wash at 0.9 µL/min, for a total method time of 8.5 min.

Data were acquired on an Orbitrap Astral mass spectrometer with FAIMS (CV –45 V) in DIA mode. MS1 scans were acquired in the Orbitrap at 240,000 resolution over m/z 390-790 (AGC 500%, max. injection time 100 ms). DIA MS2 scans were acquired in the Astral analyser using 40 × 10 m/z windows covering m/z 390-790, HCD at 25% NCE, a scan range of m/z 150-2,000 (AGC 500%, max. injection time 10 ms) and a 0.5 s cycle time.

Raw files were processed with DIA-NN Enterprise 2.6.1 (© Aptila Biotech GmbH).

SUMMARY

CoreJet™ integrated emitters enable Aurora Series® columns to deliver the robustness that large-scale tissue proteomics demands. A single Aurora® Rapid™ XT 8 cm × 75 µm column ran more than 1,200 LMD tissue injections with no clogging, no overpressure and no loss of performance. Back-pressure held steady at ~90 bar (± 5 bar) across the entire series, and peaks stayed sharp with stable retention times from the first injection to the last.

Across all 11 QC checkpoints the column delivered an average of ~3,300 protein groups and ~13,600 peptides from 50 pg HeLa, median peak widths of ~1.6-1.7 s and an average Pearson correlation above 0.98 for protein intensities. For laboratories running spatial and low-input tissue cohorts, one column can carry a full study with consistent, comparable data from start to finish.

Aurora® Rapid™ XT 8 cm × 75 µm C18 · AUR5-8075C18-XT
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