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Application Note

# Examining Nanoscale LC Reproducibility with Coupling of the ACQUITY™ UPLC M-Class System to SELECT SERIES™ Cyclic™ IMS

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This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

Nanoscale liquid chromatography (LC) and mass spectrometry (MS) is shown to exhibit excellent reproducibility in the analysis of quality control samples injected at set intervals within a large sample cohort of tryptically digested samples derived from ovarian cancer cell lines. An ACQUITY UPLC M-Class System, equipped with trapping and analytical columns, was coupled to a SELECT SERIES Cyclic IMS mass spectrometer operating in mobility enabled single pass Data Independent Analysis (DIA) mode. Results show that the nanoscale chromatograph coupled to the mass spectrometer delivered excellent retention time and signal intensity reproducibility, which are enabling factors that lead to consistent levels of protein identifications over a wide dynamic range, in addition to maintaining data integrity for statistical analyses.

## Benefits

- Reliable nanoscale chromatography

- Ion mobility resolution

- Mass spectrometer optic mode

- Dynamic range

## Introduction

Quadrupole Time-of-Flight (Q-ToF) mass spectrometers are a well-established tool for discovery proteomic experiments. These instruments display sensitivity, speed, and high mass resolution, which are important characteristics required for successful analysis of these challenging sample types.[1]

Proteomic focused research typically employs nanoscale chromatography coupled with mass spectrometry as the preferred analytical technique, especially in cases where sample amounts available for analysis are limited. In some cases, such as those experiments investigating potential biomarkers involved in disease, samples derived from a large number of donors are common. In analyzing these large sample cohorts, the requirement for reproducible and robust measurements is critical to maintain data integrity for the statistical analyses that are performed downstream of the data acquisition.

This application brief examines the use of nanoscale chromatography, using the ACQUITY UPLC M-Class System, for the analysis of standard *E. Coli* tryptic digest samples which were injected as a quality control sample within a large sample cohort, with measurements made using the SELECT SERIES Cyclic IMS operating in ion mobility enabled DIA acquisition mode. Results suggest that the liquid chromatography-mass spectrometry (LC-MS) combination delivers the required performance such that the results of the wider study are statistically relevant.

## Experimental

### Sample Description

Waters MPDS *E. Coli* tryptic digest (p/n: 186003196 <https://www.waters.com/nextgen/global/shop/standards--

) was diluted in 1 mL water to prepare a solution allowing injections of 100 ng for each injection.

## Method Conditions

LC Conditions

| | |
|---|---|
| LC system: | ACQUITY UPLC M-Class System |
| Trapping column: | Symmetry™ $C_{18}$, 5 µm, 180 µm x 20 mm (p/n: 186008821) |
| Analytical column: | HSS T3, 1.8 µm, 75 µm x 250 mm (p/n: 186008818) |
| Column temp.: | 40 °C |
| Sample temp.: | 10 °C |
| Flow rate: | 300 nL/min |
| Mobile phase A: | Aqueous 0.1% formic acid |
| Mobile phase B: | Acetonitrile + 0.1% formic acid |
| Trapping conditions: | Two minutes at 5 µL/min, 99% solvent A |
| Gradient: | 5% to 35% mobile phase B over 90 minutes |
| Column equilibration time: | 30 minutes |

MS Conditions

| | |
|---|---|
| MS system: | SELECT SERIES Cyclic IMS |
| Ionization mode: | ESI positive |
| Mass resolution: | 50,000 FWHM |
| Ion mobility resolution: | Single pass, 65 FWHM |
| Acquisition mode: | HDMS$^E$ |
| Acquisition mass range: | 50–2000 amu |
| Integration time: | 0.5 seconds |
| Reference material: | Glu Fibrinopeptide B sampled every 120 seconds |
| Capillary voltage: | 3.2 kV |
| Transfer CE, function 2: | 20–46 V |
| Cone voltage: | 30 V |

| | |
|---|---|
| Data Management | |
| MS software: | MassLynx™ |
| Data processing: | ProteinLynx Global Server, Tibco Spotfire® |
| Databases: | Uniprot *E. Coli* - reviewed sequences only |
| False discovery rate: | 4% |

# Results and Discussion

The *E. Coli* sample was analysed after every ten injections of the cancer cell line samples which resulted in a time separation of approximately 24 hours between *E. Coli* injections. A typical chromatogram for one such injection is shown in Figure 1, and the total number of injections of *E. coli* during the course of the experiment was 23. Thus, the entire experiment was just over 23 days in total.
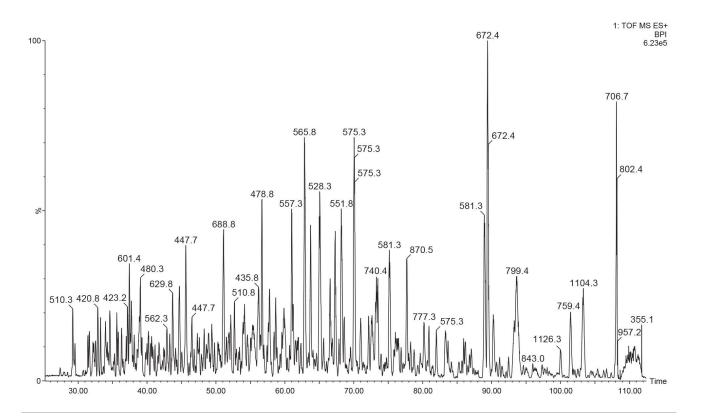


*Figure 1. Typical nanoscale chromatography for E. Coli tryptic digest sample.*

After processing and database searching each raw datafile, metrics that can be analysed included retention time, signal intensity and mass accuracy. With reproducibility and robustness demonstrated for each of these metrics, great confidence can be gained in the data obtained for the wider analytical study, especially as injections of the real samples from the wider study may be expected to have a negative impact.

Focussing on five peptides that eluted at various points across the 95-minute chromatographic elution space,

Figure 2, shows that Coefficient of Variances (CV) of 1% for retention time measurements that are routinely obtained. Extracting data for various peptides of varying intensities into a box and whisker plot, Figure 3, intensity reproducibility is also consistent with CVs of less than 20% obtained.
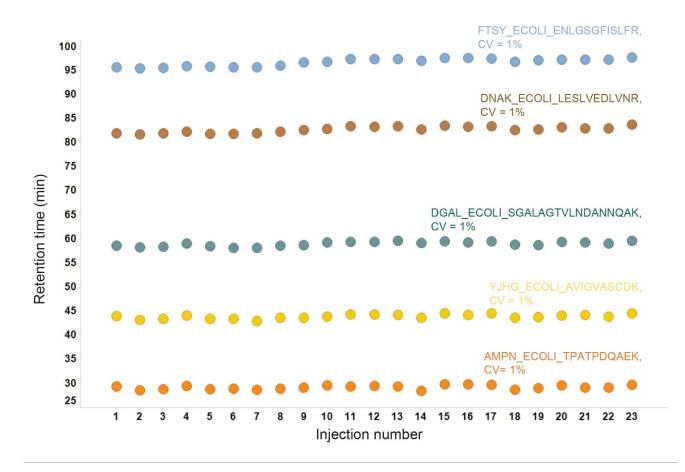


Figure 2. Retention Time reproducibility for five E. Coli peptides over the chromatographic separation space, 1% CVs routinely observed.
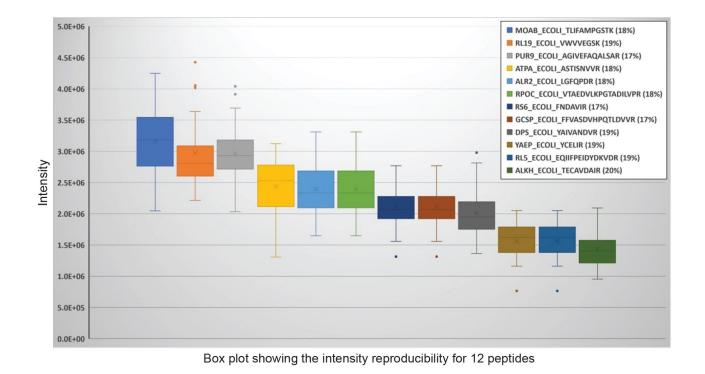
Box plot showing the intensity reproducibility for 12 peptides

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*Figure 3. Signal Intensity reproducibility for 12 E. Coli peptides, 20% CVs routinely observed.*

The mass spectrometer performance is highlighted with a distribution of the mass accuracy of 275,000 peptide mass measurements derived from all the search results, Figure 4, with 82% found to be within +/-2 ppm of their theoretical mass to charge value.
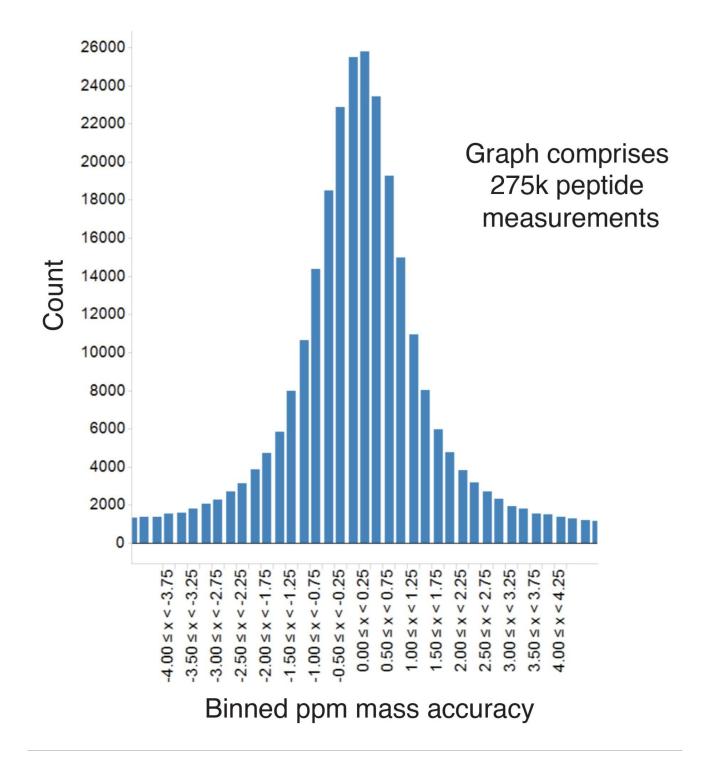
Figure 4. Mass accuracy measurements for 275k peptides, 82% within +/- 2ppm.

The database search results given to protein and peptide identification rates derived from database search results are important for the consistency of measurements, particularly when considering injctions within a larger study. Highlighted in Figure 5 are plots of these across the experiment, with protein identification rates of around 1,100 and peptides numbering around 22,000 for each injection. Protein sequence coverage is a measure of the reproducibility of peptide identifications and Figure 6 highlights twelve proteins with 55% to 93% coverage but with the Coefficient of Variances of these ranging from 3 to 5%. Excellent reproducibility of protein identifications is observed, where the same 1000 identifications are observed in at least 17 of the 23 injections, Figure 7. The sum of the intensities of fragment ions associated with these protein identifications are found to span four orders of intensity dynamic range, Figure 8.
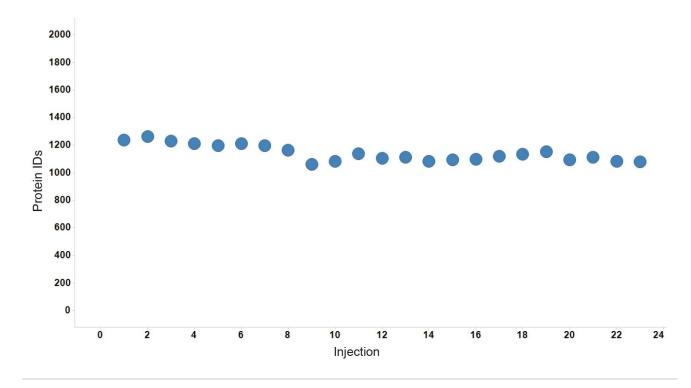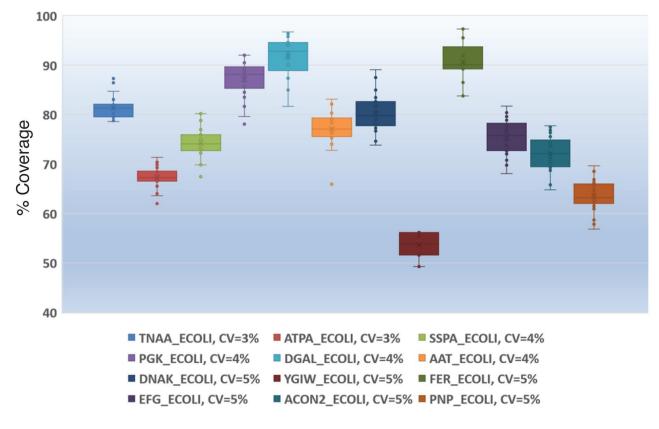


*Figure 5. Protein identifications for each of the 23 injections.*

Box plot showing the sequence coverage reproducibility for 12 proteins

*Figure 6. Sequence Coverage reproducibility for 12 E. Coli proteins, 5% CVs are routinely observed.*
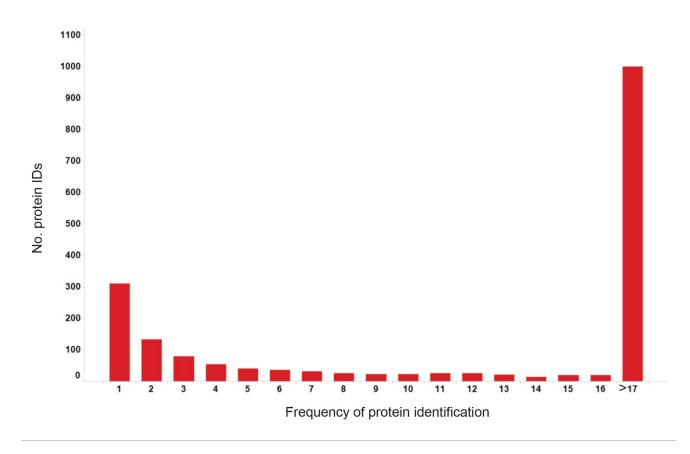
*Figure 7. Protein and peptide identification reproducibility, 1,000 appearing in 75% of injections.*
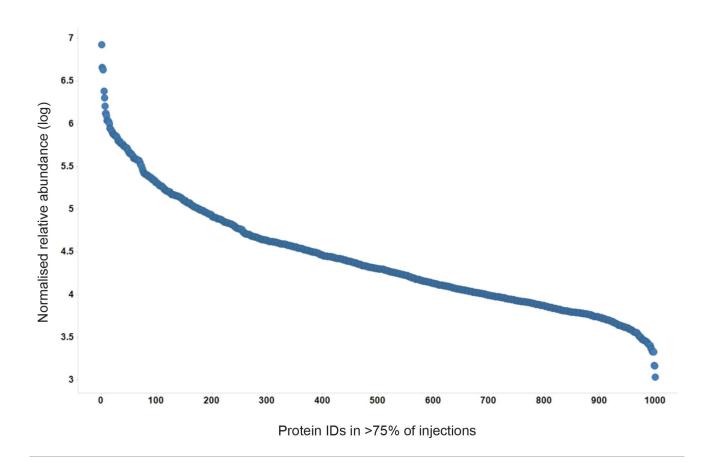
*Figure 8. In-sample dynamic range representing proteins identified in >75% of injections are shown to cover 4 orders of dynamic range.*

## Conclusion

An ACQUITY UPLC M-Class System equipped with nanoscale columns coupled to a SELECT SERIES Cyclic IMS Mass Spectrometer has been demonstrated to deliver excellent retention time, signal intensity reproducibility and mass accuracy. These data were derived from the analysis of data from a quality control standard injected over the course of a 23-day experiment. The robustness of the measurement of these factors lead to consistent levels of protein identifications over a wide dynamic range of signal intensities and reinforced the statistical relevance of the wider ovarian cancer sample measurements.

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## References

1. Chris Hughes, Lee A. Gethings, Robert S. Plumb, Qualitative and Quantitative Performance of Cyclic IMS in Nanoscale Proteomic Experiments'.  Waters Application Note, 720007381 2021.

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## Featured Products

ACQUITY UPLC M-Class System <https://www.waters.com/134776759>

SELECT SERIES Cyclic IMS <https://www.waters.com/waters/nav.htm?cid=135021297>

MassLynx MS Software <https://www.waters.com/513662>

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