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Application Note

MaxPeak High Performance Surfaces Enable Reproducibility and Enhanced Experimental Robustness in High Throughput Proteomics

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Abstract

Robust and reproducible LC-MS is an essential requirement in large scale clinical proteomic experiments, and the available sample amounts lend themselves to the use of larger I.D. column geometries compared to traditional proteomic experiments.

A quality control sample of a complex tryptic digest was injected nearly two hundred times onto a MaxPeak High Performance Surfaces Technology enabled LC system, the ACQUITY PREMIER, fitted with a CSH 2.1 mm I.D. column and eluent coupled to a SYNAPT XS Mass Spectrometer. The injections of an *E. coli* digest onto the CSH column took place at set intervals inside a larger clinical proteomic study. The raw and processed data is used to showcase excellent reproducibility and robustness of several characteristics of the liquid chromatograph (LC) and mass spectrometer (MS) used in the experiment – information which is important for both the QC of the study and applicability to wider clinical proteomic cohorts.

Chromatographic parameters; peak width and retention time reproducibility, are assessed across all the injections with over 800 protein and over 1 million individual peptide measurements contributing. The consistency of measured peptide intensities and protein sequence coverage leads to a large percentage of total proteins being identified across an experiment which spanned over a six week period.

Benefits

- HPS technology removes any issues with metal adsorbed species
- Routine, robust, and reproducible analytical scale LC-MS
- Suitable for large cohort, clinical proteomic analyses

Introduction

Increased interest in the field of clinical proteomics, where sample amounts are not limited, has led to various studies into the utilization of larger scale chromatography than would typically be used for more traditional, nanoflow-based proteomic experiments. Larger scale chromatography tends to be more user friendly, robust, and reproducible than the traditional nanoscale proteomics set up[1] which can be sometimes seen as only accessible to an expert user.

Waters developed a class of new technologies, known as MaxPeak High Performance Surfaces (HPS), and these have been applied in the development of a new liquid chromatographic system called the ACQUITY PREMIER.[2] While primarily aimed at limiting adsorption of certain compounds to metal surfaces and thus enhancing their detection, investigations suggest that a user may be completely unaware they are losing information from any sample being analyzed. This is particularly concerning in untargeted, discovery type analyses and it therefore follows that the use of MaxPeak HPS Technology is preferable for any experimental procedure.

A large-scale plasma proteomic experiment of over 500 samples, injected three times each, was carried out and during this analysis, after every 8 samples, a quality control injection of *E. coli* tryptic digest was performed. By analyzing these QC results, it is possible to derive metrics for the reproducibility and robustness of the entire experiment. This application brief aims to show that over the six-week duration of the study, outstanding reproducibility and robustness, both chromatographic and mass spectrometric, is observed.

Experimental

Sample Description

A sample of 2 µg MPDS *E. coli* (186003196 <https://www.waters.com/nextgen/us/en/shop/standards--%20reagents/186003196-massprep-e-coli-digest-standard.html>), was injected every 4 hours in-between injections of plasma tryptic digests that were being analyzed for the wider scale clinical proteomics experiment. Throughout the six week experiment, the *E. coli* sample was injected over 180 times and processed using Progenesis QIP, PLGS, and data viewed with Spotfire data visualization software.

LC Conditions

LC system(s):	ACQUITY PREMIER (HPS)
Column(s):	ACQUITY UPLC CSH 2.1 mm x 100 mm (HPS)
Column temp.:	55 °C
Sample temp.:	8 °C

Flow rate:	150 µL/min
Mobile phase A:	Water + 0.1% formic acid
Mobile phase B:	Acetonitrile + 0.1% formic acid
Gradient:	1 to 35% B in 16 mins followed by re-equilibration

MS Conditions

MS system:	SYNAPT XS
Ionization mode:	Electrospray Positive Ion
Acquisition mode:	ToF HDMS^{E}
Acquisition range:	50–2000 Da
Collision energy:	Transfer CE ramp using Look Up Table[3]
Capillary voltage:	2.2 kV
Cone voltage:	30 V
Lock mass solution:	Glu Fibrinopeptide B (2+, *m/z* 785.8426)

Data Management

Chromatography software:	MassLynx v4.2
MS software:	MassLynx v4.2
Informatics:	Progenesis QI for Proteomics (PQIP), PLGS

3.0.3, Spotfire (Tibco)

Results and Discussion

Reproducibility – Chromatography

Figure 1A shows the raw data chromatograms from two injections (30 days apart), with Figure 1B representing a retention time window from 5 to 8.5 minutes. Figure 2, the distribution of chromatographic peak widths of all identified peptides, shows the majority eluting over 3 seconds FWHM. Further to this, Figure 3 represents the frequencies of retention time measurements for a selection of precursor ions from all the injections associated with tryptophanase (P0A853; TNAA_ECOLI). Coefficient of variation (CV) of 1% or less are observed, showing clearly that excellent chromatographic retention time reproducibility has been maintained across the experiment.

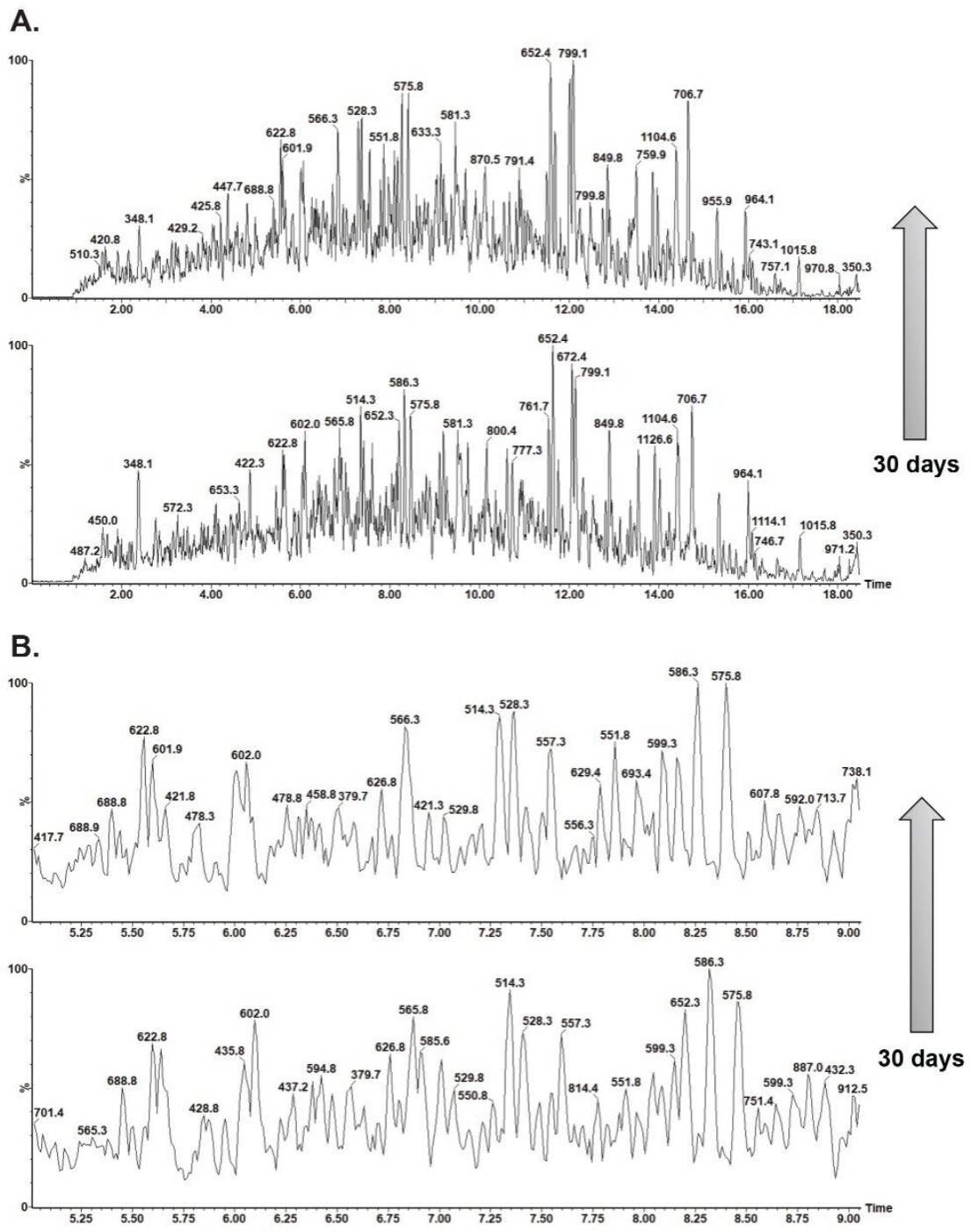

Figure 1. (A) Raw data Base Peak Ion (BPI) chromatograms from two injections of 2 µg E. coli performed approximately 30 days apart. (B) Raw data BPI chromatogram zoomed into a narrower retention time window.

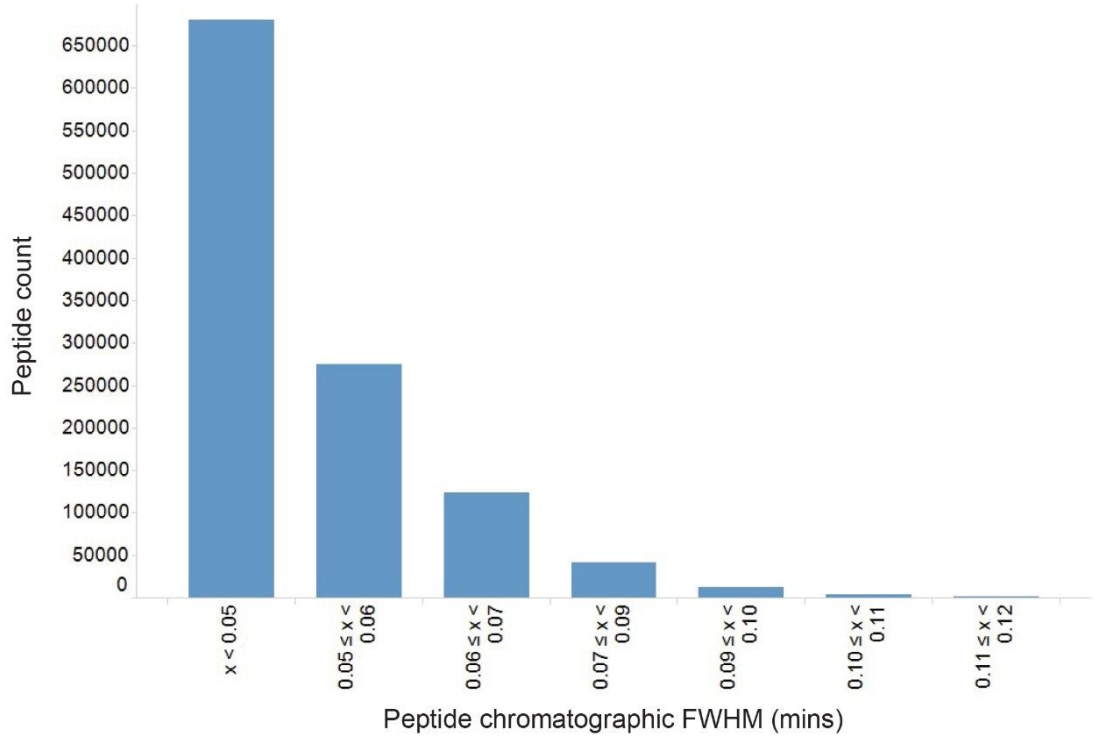

Figure 2. Identified peptide precursor chromatographic peak width distribution. The data comprises >1 million individual measurements from the entire experiment.

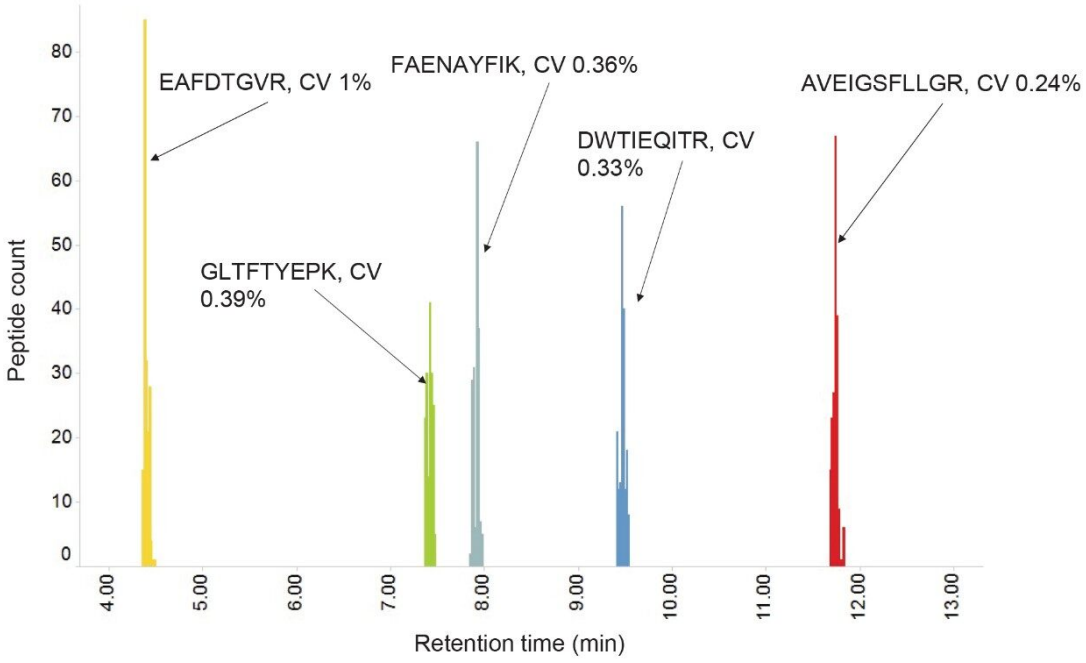

Figure 3. Frequencies of retention time measurements for 5 selected precursor ions associated with tryptophanase. Co-efficient of variation of measurements for each peptide are highlighted.

LC-MS Robustness – Peptide Intensities and Mass Accuracy

One of the outputs generated from PQIP and PLGS processing for identified proteins and peptides is the sum of product ion intensities for each peptide and can be used as a means of monitoring intensity robustness. The results for three *E. coli* related proteins are shown in Figure 4, with ions from the most intense protein tryptophanase (TNAA_ECOLI) and two less intense proteins, malate dehydrogenase (P61889; MDH_ECOLI), and aldehyde-alcohol dehydrogenase (P0A9Q7;ADHE_ECOLI), shown. Further evidence of the robustness of the experiment is given by the peptide precursor mass accuracy, where it is found that >95% of peptides are measured within ±5 ppm of their theoretical value, Figure 5.

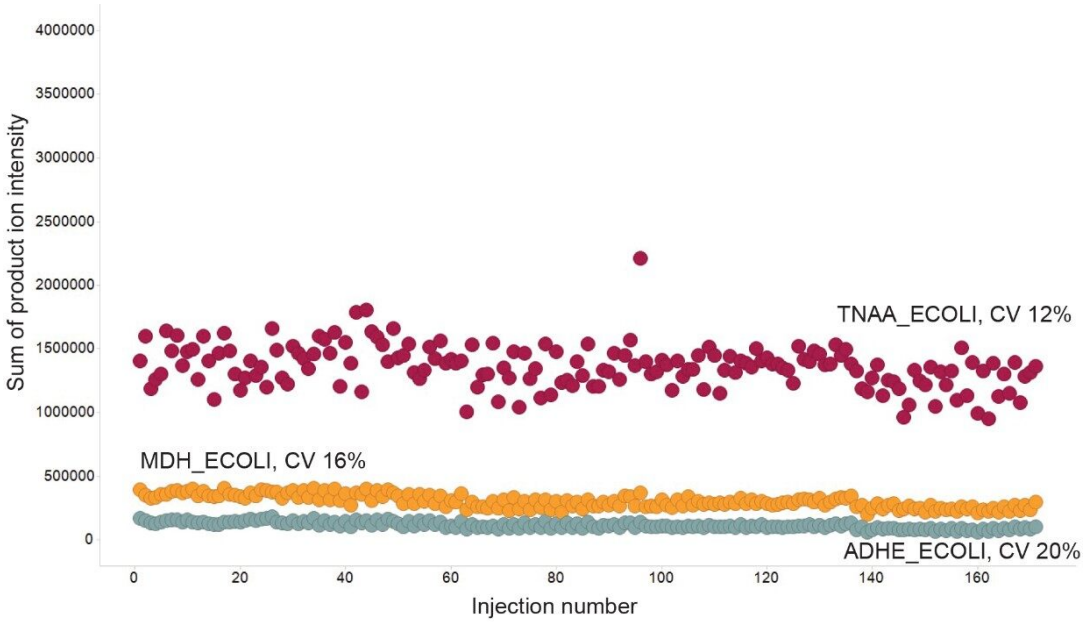

Figure 4. Sum of product ion intensities for peptides for three proteins against injection number to monitor intensity robustness across the experiment.

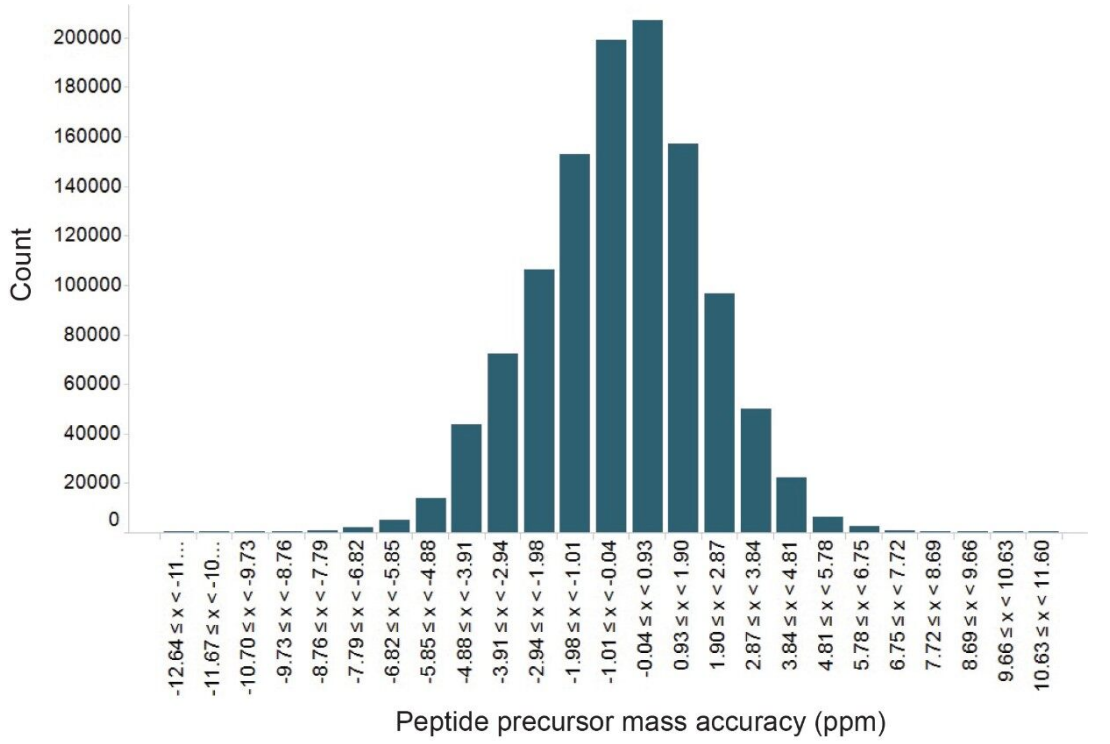

Figure 5. Frequencies of peptide precursor mass accuracy.

MS Robustness – Protein Sequence Coverage and Identifications

One parameter derived is the protein sequence coverage resulting from peptide identifications for each protein. For one protein TNAA_ECOLI, an average sequence coverage of 76% (CV 2.8%) is observed across the experiment, Figure 6A. Figure 6B is a distribution graph showing the ranges of the sequence coverage for all identified proteins and shows a modal range of 40 to 49%. The goal of many proteomic experiments is to first identify proteins prior to determining any regulation of these when comparing different samples/conditions. However, for these QC analyses where the same sample has been injected, the reproducibility of identifications throughout the five-week duration has been measured. Figure 7 shows a Venn diagram whereby identifications comprised of three injections, which correspond to the early (5 days), middle (18 days), and late (38 days) stages of the experiment are combined and compared. This suggests that approximately 76% of proteins are identified in injections across the whole experiment.

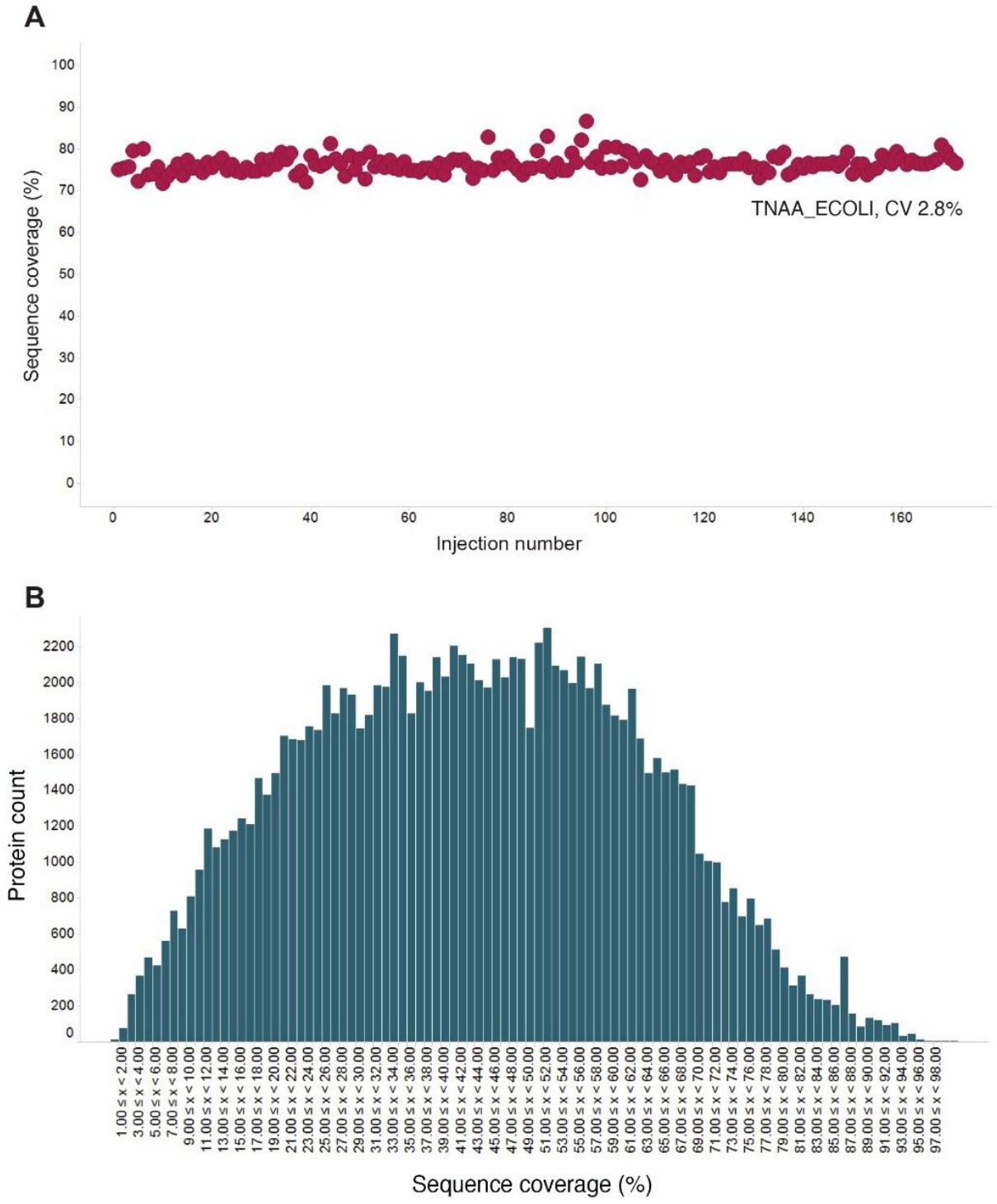

Figure 6. (A) Sequence coverage reproducibility for the protein tryptophanase. (B) Sequence coverage distribution for all proteins identified in the experiment.

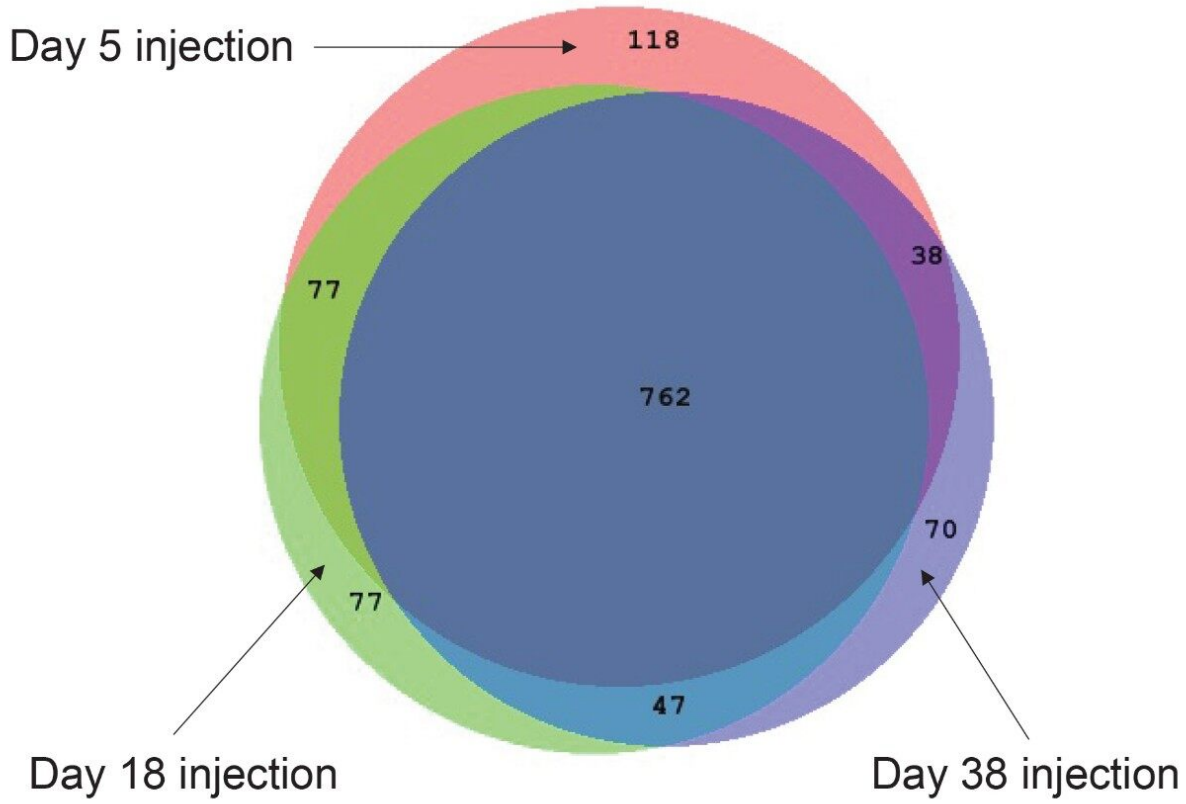

Figure 7. E. coli protein identifications from injections at various stages of the experiment.

Conclusion

MaxPeak HPS Technology ensures that adsorption to column hardware material is minimized. The routine nature of analytical scale chromatography suggests an experimental avenue when sample limitations are not an issue, allowing for high throughput analyses. We have shown results from analyzing a QC sample injected within a large scale proteomic experiment, utilizing an LC system and column that have been treated with MaxPeak High Performance Technology, and have shown this LC-MS configuration with SYNAPT XS to exhibit excellent robustness and reproducibility. Over the course of a six week LC-MS study, data metrics derived from the interspersed QC's demonstrate excellent reproducibility of peptide intensities and precursor retention times. Resulting protein identifications and their sequence coverage is also shown to be maintained throughout the study.

References

1. Lennon, S, Hughes, CJ, Muazzam, A, Townsend, PA, Gethings, LA, Wilson, ID, Plumb, RS. High-Throughput Microbore Ultrahigh-Performance Liquid Chromatography-Ion Mobility-Enabled-Mass Spectrometry-Based Proteomics Methodology for the Exploratory Analysis of Serum Samples from Large Cohort Studies. ***J. Proteome Res***, 10.1021/acs.jproteome.0c00821.

2. Mathew DeLano, Thomas H. Walter, Matthew A. Lauber, Martin Gilar, Moon Chul Jung, Jennifer M. Nguyen, Cheryl Boissel, Amit V. Patel, Andrew Bates-Harrison, Kevin D. Wyndham. ***Anal. Chem***. 2021, 93, 5773−5781.

3. Distler, U, Kuharev, J, Navarro, P, Levin, Y. Drift Time-Specific Collision Energies Enable Deep-Coverage Data-Independent Acquisition Proteomics. ***Nature Methods***, volume 11, pages 167–170 (2014).

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