

A novel nano UHPLC system for high robustness and reproducible proteomics analysis

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Introduction

Mass spectrometry (MS)-based proteomic workflows depend on efficient peptide separation to reduce sample complexity prior to ionization and mass spectrometric detection. The separation step is critical for minimizing ion suppression and enhancing proteome coverage depth. Reversed-phase nano-liquid chromatography (nano-LC) using C18 columns is the most widely used technique for this purpose. Peptides are separated based on their hydrophobicity, either through direct elution from the analytical column (1D-LC) or following pre-concentration and desalting on a trap column (2D-LC).

Key challenges associated with nano-LC separations include: (1) minimal carry over to reduce the risk of cross-contamination and maintain data integrity across sequential runs, (2) low overall solvent consumption, (3) minimal overhead time for high sample throughput, (4) high gradient precision for optimal peptide separation and run-to-run consistency, (5) a tool that provides real-time feedback on instrument performance, and (6) overall robustness and reliability of the platform.

1. Sample carry over

Our new UHPLC system proteoElute integrates a new, customizable washing procedure with optimized wash cycle methods that can be tailored to different analytical application. The updated injection system helps boosting sensitivity for low-abundance analytes by reduction of background contamination from previous injections — especially important for challenging applications like plasma proteomics and single-cell proteomics.



Fig. 1. Carry over evaluation from previous injections based on MS1-level quantification

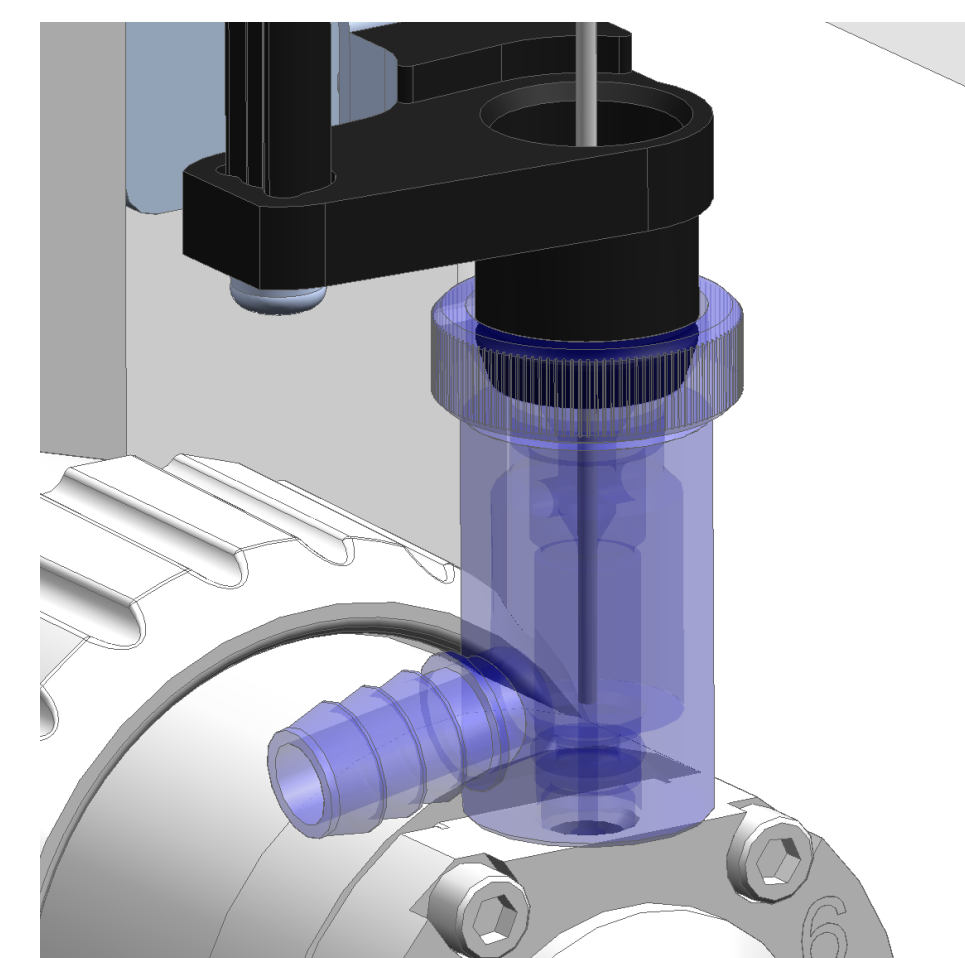


Fig. 2. New injection port design that allows more efficient wash

2. Solvent consumption

Lower solvent consumption translates to overall lower costs, reduced solvent waste and longer intervals without the need to refill solvent bottles. Evaluations using default settings show that total solvent consumption per injection is reduced by ~50% compared to the previous generation of systems.

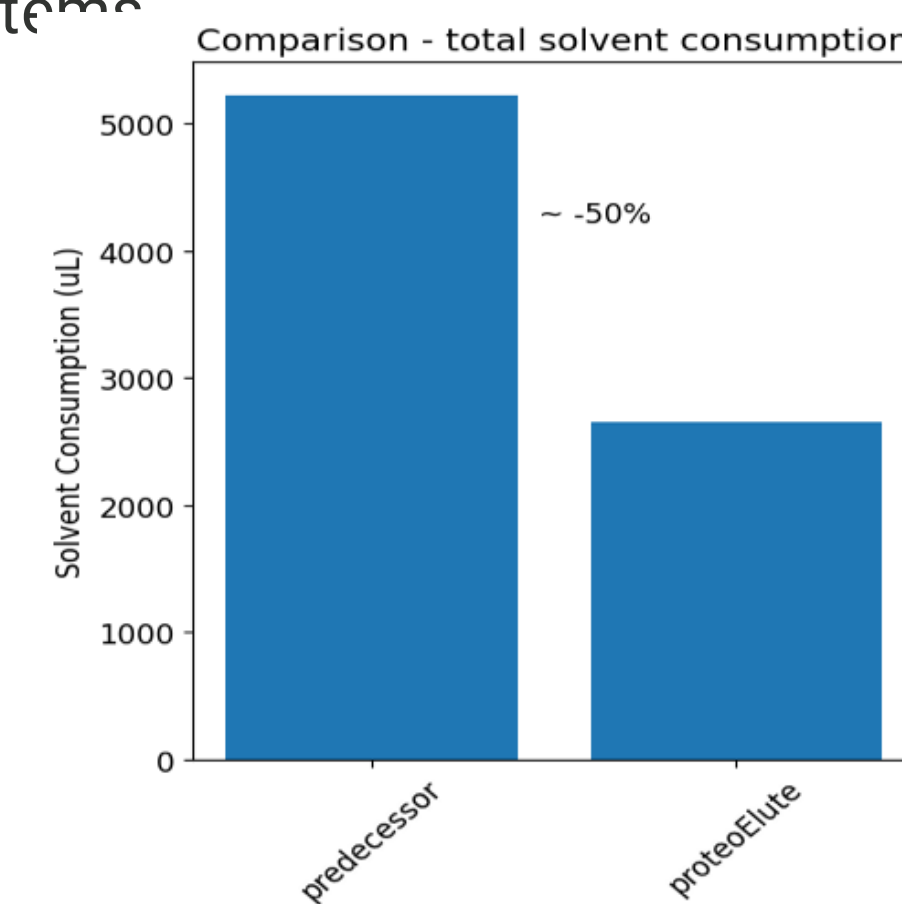


Fig. 3. Comparison of solvent consumption of the previous generation LC system with proteoElute

4. Gradient precision

Gradient formation can be monitored using iRT peptides, providing a reliable check across gradients of varying lengths. Consistency and proper performance was confirmed with this approach for proteoElute LC system.

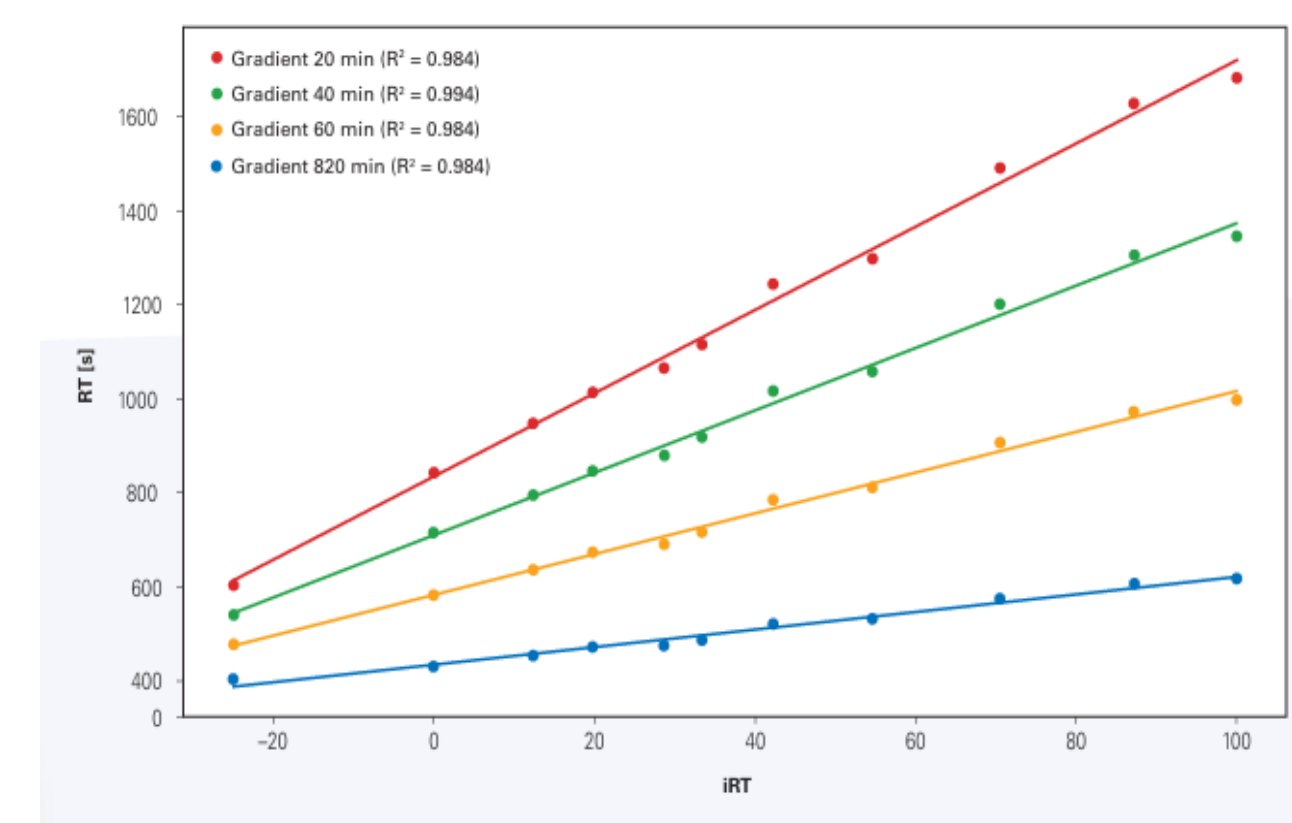


Fig. 4. iRT peptides were injected onto a PepSep Advanced column and eluted by a linear gradient from 0-90% acetonitrile with 0.1% formic acid. Retention times plotted against iRT values and fit by linear regression

3. Sample throughput

While the gradient cannot be shortened without affecting peptide elution and separation performance, overhead time could be reduced by introducing a new scheme for equilibration, sample loading, and initial gradient formation.

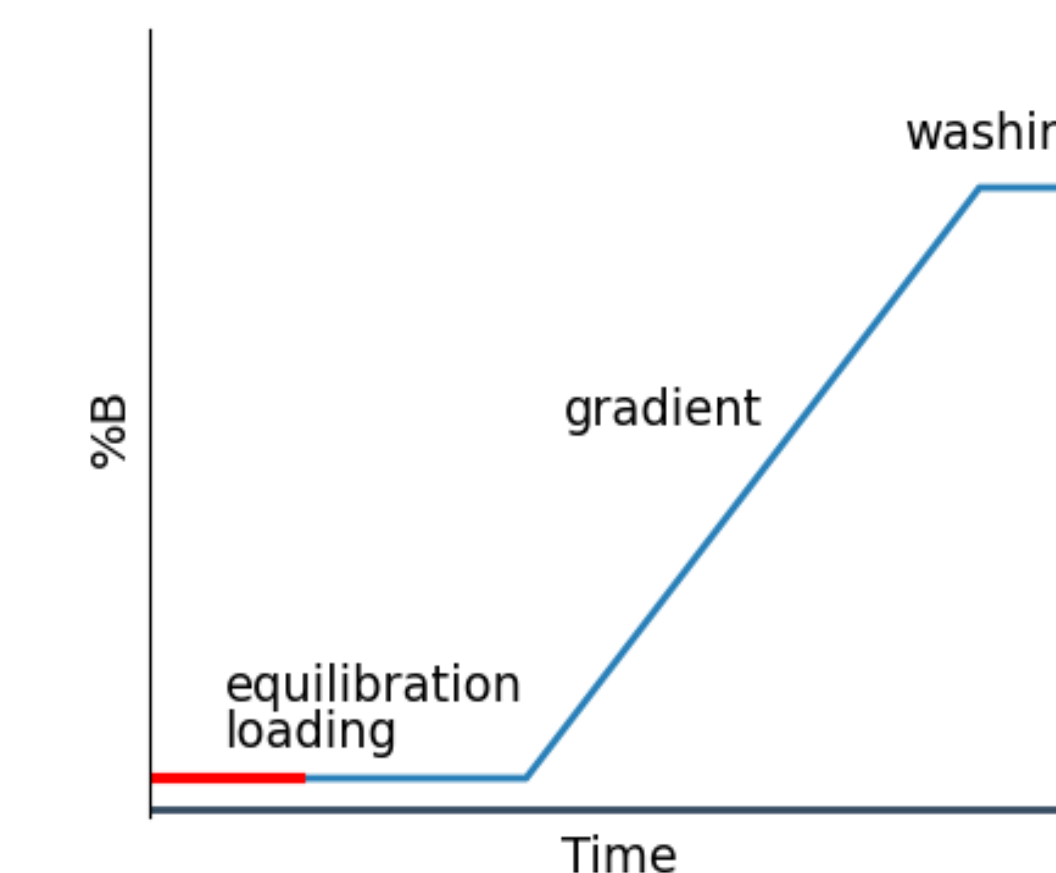


Fig. 5. Accelerated sample loading scheme. Injection time saving indicated in red.

5. Real-time monitoring

Full integration with TwinScope, a new cloud-based software tool that allows to monitor and store system data over time, providing total assurance in the performance of the instrument.

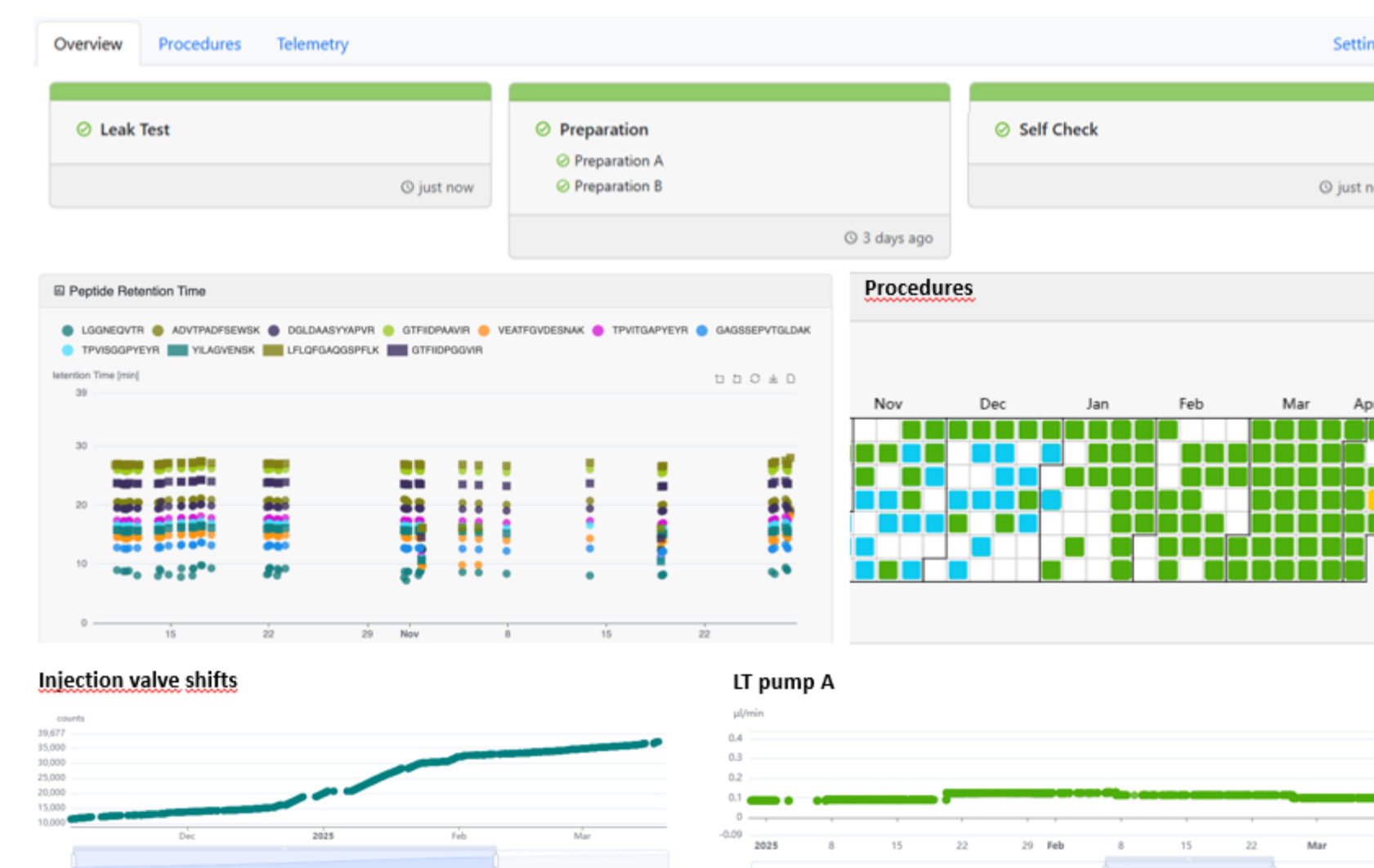


Fig. 6. Extracts from the TwinScope web interface. From top to bottom: system overview, iRT retention time plot, system status with traffic lights, injection valve shifts, leak test pump A

6. Improved robustness

Upgrades in hardware, along with additional diagnostic procedures and automatically scheduled self-check routines were introduced to enable early detection of potential issues. In combination with TwinScope, this helps to ensure reliable performance.

Conclusion

- Reduction of carry over at reduced solvent consumption, with parameters to fine-tune the washing procedure
- Increased sample throughput by reduction of column equilibration and sample loading time
- Real-time monitoring and enhanced system robustness for greater reliability, consistency, and overall operational efficiency

Technology