From discovery to application: Enabling scalable, reproducible targeted proteomics through standardized Evosep worfklows

Iulia Macavei, Magnus Huusfeldt, Stine R. Rasmussen, Maurine Fucito, Stoyan Stoychev, Nicolai Bache, Dorte B. Bekker-Jensen

Evosep Biosystems, Denmark

Highlights

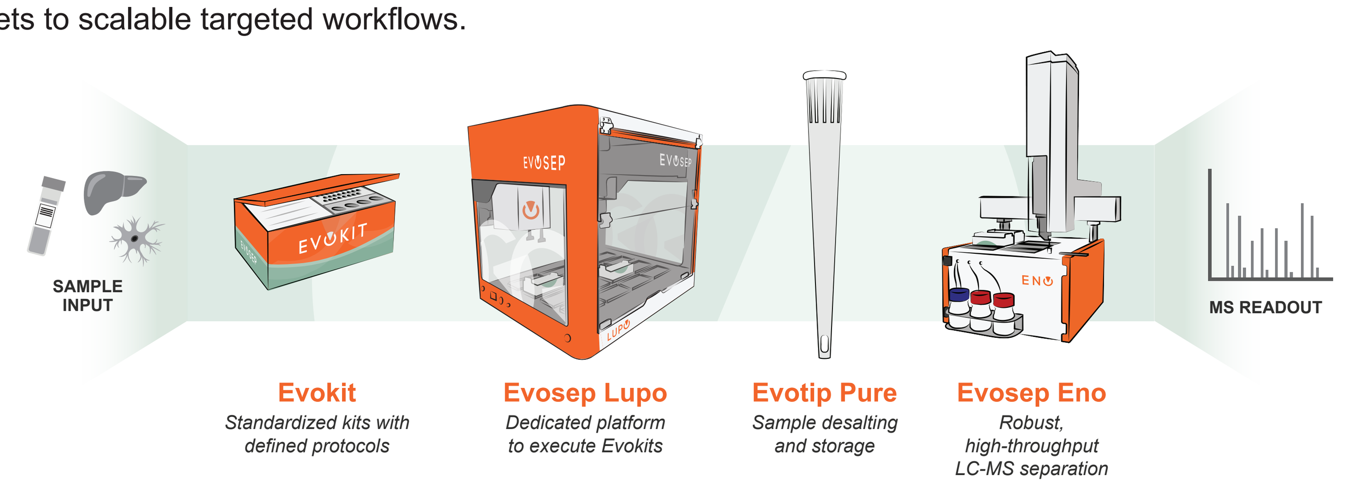
- Evosep Proteomics enables a high-performance Sample to Separation solution for standardized and scalable mass spectrometry.
- Robust ecosystem for scalable Proteomics with retention time reproducibility below 1 s RT SD and peptide CVs below 5% using the Evosep Eno 200 SPD method.
- Highly robust assay sensitivity from Evokits with reproducible quantification from sub-nanogram to microgram protein lysate input levels.

From discovery to targeted application

From discovery to application through a standardized ecosystem

Despite the large number of identified biomarker candidates, only a limited subset has been translated into robust assays for clinical research. Targeted LC–MS enables this transition by converting discovery-derived targets into quantitative verification and validation workflows. Achieving this at scale requires standardized and controlled processes that ensure consistent execution across samples and studies.

Evosep Proteomics addresses this by integrating sample preparation and LC–MS into a unified ecosystem. Evokits, Evosep Lupo, and Evosep Eno form a seamless workflow, with Evotip Pure linking preparation to analysis. Together, these components operate as a single controlled system, enabling consistent transfer from discovery datasets to scalable targeted workflows.



Reproducible results with built-in controls and walk-away automation

The workflow enables automated processing from protein extract to purified digest loaded on Evotip using Evokits, which include all required reagents, consumables and Evotips. Execution on Evosep Lupo incorporates built-in traceability and quality controls, while predefined workflows and software interface minimize user intervention. Integrated QC samples monitor both sample preparation and LC–MS performance throughout the study. Defined peptide panels track digestion efficiency, workflow recovery, and instrument stability, ensuring controlled and reproducible targeted analysis.

SETUP — Handling time: ~5 min
- Standardized Evokit
- Guided setup on Evosep Lupo
- Integrated checks
- Certificate of Analysis
- Direct sample list import

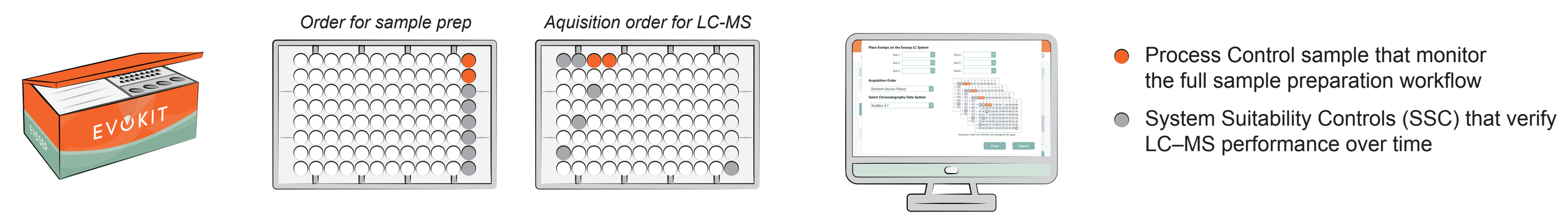
RUN — Handling time: 0 min
- Uninterrupted run with built-in QC
- Real-time countdown

OUTPUT — Handling time: ~5 min
- Evotips ready for analysis or stable storage
- MS-ready sequence list generated
- Logfile with full traceability

Sample preparation at scale with Evokits and Evosep Lupo

Built-in quality controls incorporated with each Evokit

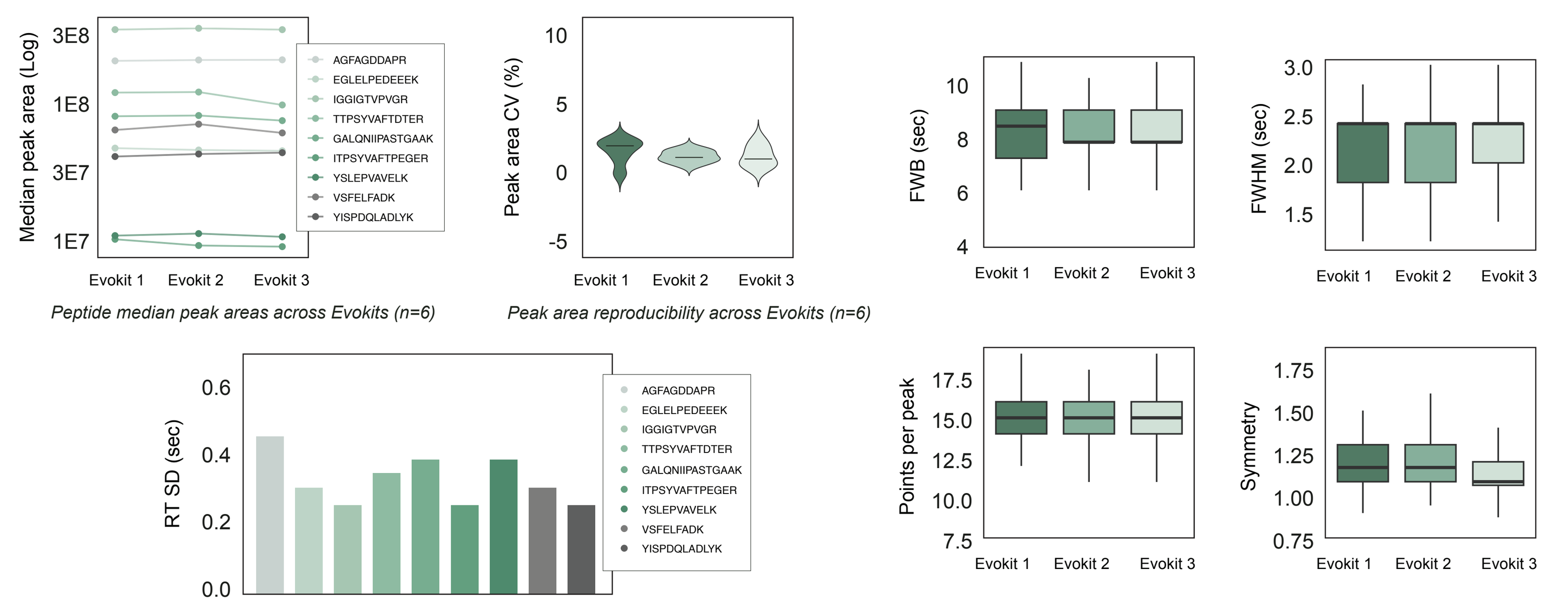
Each Evokit Digest includes both process control samples and system suitability controls, which are used to monitor the sample preparation and to verify LC–MS performance over time. This setup enables continuous quality assessment of pre-analytical handling and instrument stability throughout the analytical process.



Each Evokit Digest includes process control and System Suitability Control samples to monitor the sample preparation and LC-MS performance over time.

System Suitability Control monitors LC-MS performance

Nine reporter peptides were used to monitor the performance of quality control samples processed with three different Evokits with Evosep Lupo, using a Waters Xevo TQ-Absolute XR MS. The SSC samples assessed the LC-MS performance on the Evosep Eno instrument by tracking chromatography and signal stability across Evokits. Retention time, peak shape, peak width, and signal intensity remained consistent across Evokits, injections, and days, with low RT variability (<1 s) and peptide CVs <5%, demonstrating stable and reproducible instrument performance with the 200 SPD method.



Peptide median peak areas across Evokits (n=6)

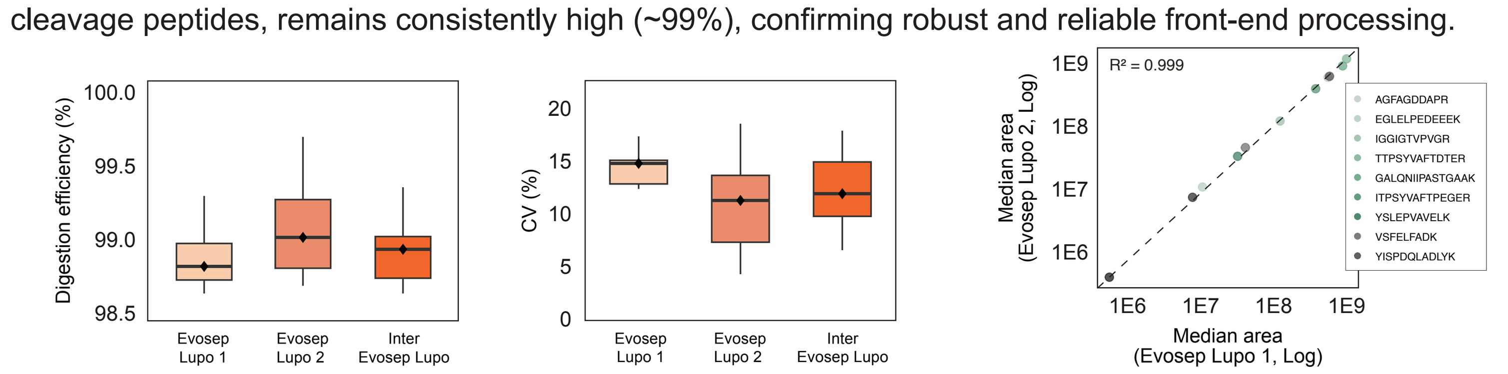
Peak area reproducibility across Evokits (n=6)

RT reproducibility across Evokits (n=18)

Peak metrics across Evokits, 200 SPD, MRM

Process Control Digest monitors workflow performance

The Evokit workflow on Evosep Lupo demonstrates high technical reproducibility, with concordance across systems over the full dynamic range. While the LC-MS variability remains below 5%, the overall workflow Evokit and Evosep Lupo variability (~10–15% CV) highlights excellent performance across the complete sample preparation and analysis pipeline. Digestion efficiency, calculated from the ratio of fully cleaved to missed cleavage peptides, remains consistently high (~99%), confirming robust and reliable front-end processing.

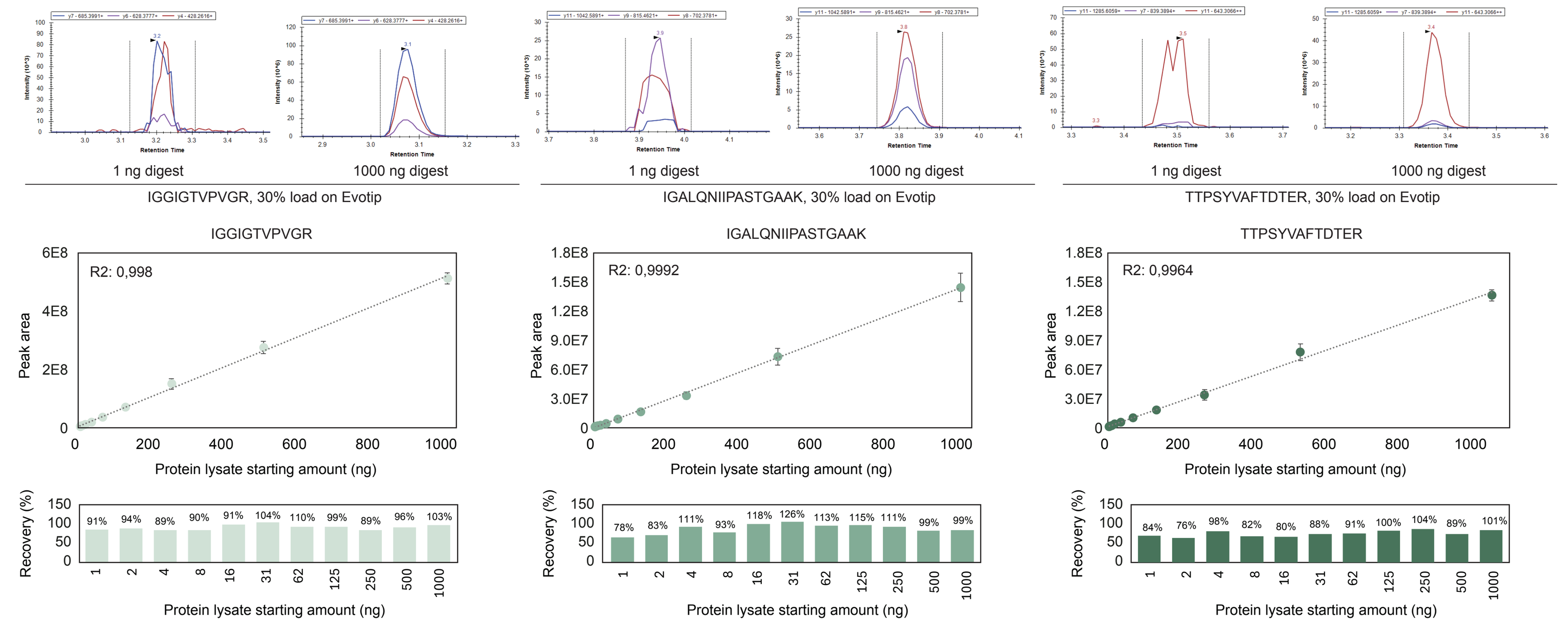


Intra- and inter- Evosep Lupo performance evaluation using digestion efficiency, reproducibility and recovery as metrics (Evosep Lupo 1: n=16, Evosep Lupo 2: n=8). Evosep Eno 200 SPD, Waters Xevo TQ-Absolute XR

Controlled, consistent results across every workflow step

High sensitivity for targeted workflows

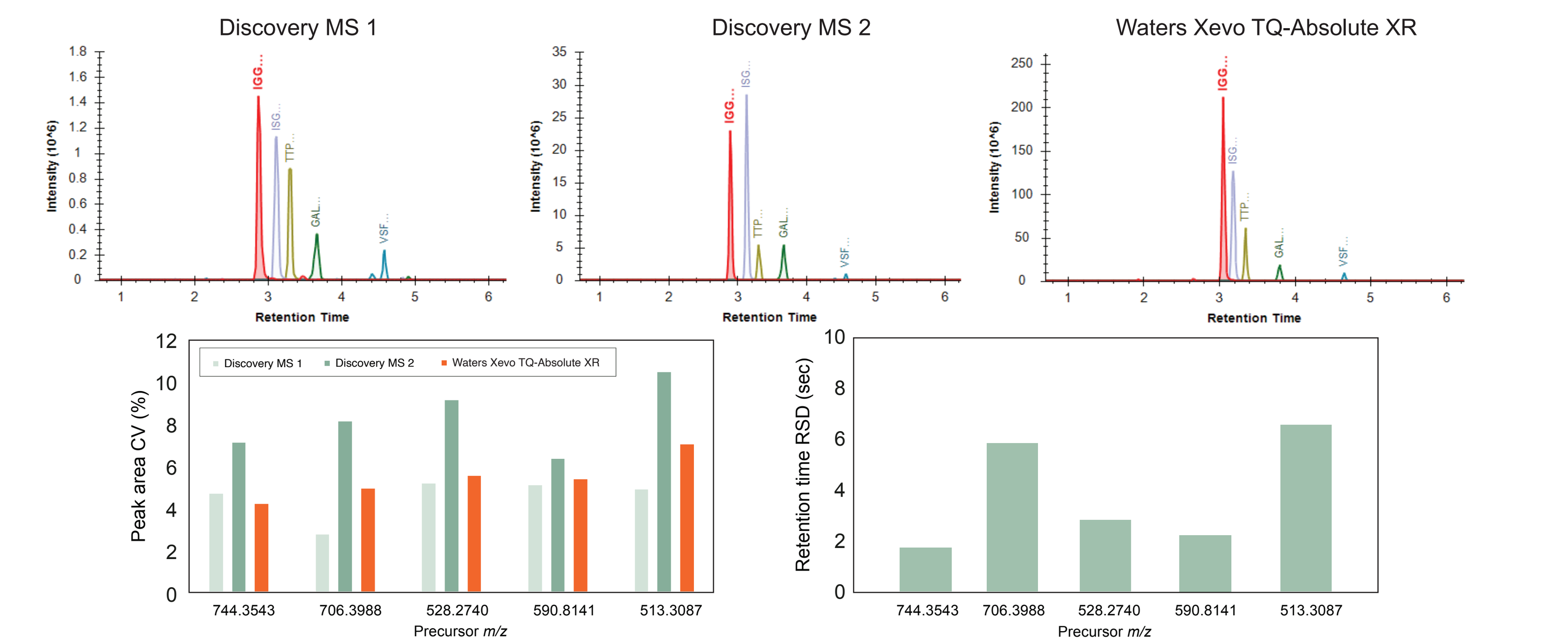
The sensitivity of the Evokit Digest was measured in MRM mode. Extracted ion chromatograms and calibration curves show high linear signal response across ~3 orders of magnitude (R^2 ≈ 0.996–0.999) using 1 to 1024 ng protein lysate with the Evokit, with 30% of the digest loaded on the Evotip for analysis. Notably, great quantification is obtained with very little starting material, demonstrating robust workflow sensitivity. The recovery remains within ~80–120% across the range, confirming accurate and reproducible quantification from sub-nanogram to microgram levels.



Sensitivity of Evokit Digest is shown with three different peptides, measured on a Evosep Eno 200 SPD, coupled to a Waters Xevo TQ-Absolute XR (n=8). Extracted ion chromatograms (top), calibration curves (middle) and recovery (bottom).

Seamless transition from discovery to targeted analysis

Evosep Proteomics enables a seamless transition from discovery to targeted analysis. This is facilitated by standardized sample preparation with Evokits using the Evosep Lupo, reproducibly generating consistent peptide features across studies as well as LC-MS analyses. Three independent digests prepared on different days were analyzed using two discovery MS systems and one QQQ MS, each coupled to a different Evosep Eno instrument. Across all conditions, the same peptide features were consistently detected with high precision (CV <10%) and retention time stability (RSD), demonstrating robustness independent of sample preparation day, LC setup, and MS platform.



Extracted ion chromatograms, workflow precision and retention time reproducibility across the Discovery and Targeted MS platforms on different days, Evosep Eno 200 SPD. 1 µg K562 lysate digested with Evokit. 30% peptide loaded on Evotip (n=6).