



Application Note EVOSEP

Evosep Proteomics - one standardized workflow from biomarker discovery to targeted verification

Highlights

- Same sample prep and LC method for discovery and targeted MRM assays.
- $R^2 > 0.99$ and 86–111% accuracy for endogenous peptides by MRM.
- Built-in controls separate LC-MS from sample preparation variability.

1. Introduction

Protein signatures are increasingly used throughout drug development to support target engagement studies, investigate mechanism of action, and monitor pharmacodynamic (PD) responses. While discovery proteomics can identify candidate biomarkers from thousands of proteins, PD studies require targeted assays capable of delivering accurate and reproducible quantification across large sample cohorts.

Translating biomarkers from discovery to targeted analysis often requires changes in sample preparation, chromatography, or mass spectrometry platforms. These changes increase method-development effort and introduce the risk that

peptide retention times, detectability, or quantitative performance will not transfer between workflows.

The Evosep Proteomics ecosystem addresses this challenge by combining Evokit™, a standardized sample preparation solution, with Evosep Eno™, a standardized liquid chromatography system, to deliver consistent sample preparation and chromatography across both discovery and targeted LC-MS applications. Evokits are processed on Evosep Lupo™ using automated sample preparation with integrated process controls. Together, these technologies support the transition of biomarker candidates from discovery proteomics into targeted pharmacodynamic assays within a unified workflow.

Here, we use a dilution series of human K562 cell line lysate to demonstrate a discovery-to-targeted workflow on the Evosep Eno, with discovery on a SCIEX ZenofTOF 8600 and targeted multiple reaction monitoring (MRM) verification on a SCIEX 7500+ (Figure 1).

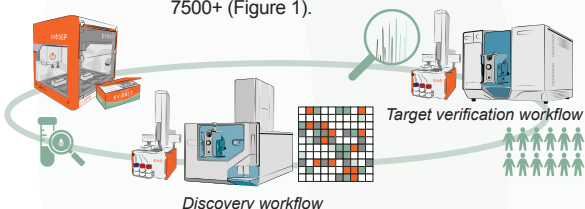


Figure 1: Standardized discovery-to-targeted workflow with Evokits on Evosep Lupo and the Evosep Eno.

2. Method details

K562 cell lysate was processed using Evokit Digest, 1x96 (EV3802, Evosep) together with built-in process control digest (PCD) and system suitability control (SSC) samples on an Evosep Lupo (EV3000, Evosep). For discovery experiments, 500 ng protein input was used and 30% of the digest was subsequently loaded onto Evtips. For targeted analysis, a dilution series of 1 to 500 ng protein input was aliquoted into the Evokit sample plate, and 30% of each digest was loaded onto Evtips.

LC-MS/MS analyses were performed at 200 SPD using the Evosep Eno with an Evosep Performance column (EV1182, Evosep) maintained at 40 °C.

Discovery analysis was performed on a SCIEX ZenoTOF 8600 with an OptiFlow Pro source operated at 4500 V and 200 °C (curtain gas 40 psi, GS1 20 psi, GS2 60 psi). Data were acquired using ZT Scan DIA with a 5 Da Q1 window and 10 ms MS/MS accumulation time over m/z 400–900. Raw files were analyzed with DIA-NN (v1.9.2) in library-free mode at 1% FDR against the reviewed human proteome (UniProt, Oct 2020, 20,600 entries) using trypsin/P with up

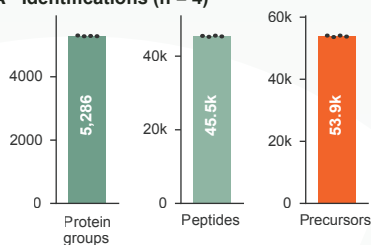
to two missed cleavages, and default settings. Conditions were searched separately, with match between runs enabled within replicates.

Targeted analysis was performed on a SCIEX 7500+ with an OptiFlow Pro source operated at 4500 V and 270 °C (curtain gas 40 psi, GS1 40 psi, GS2 50 psi). Data were acquired in MRM mode with unit resolution for Q1 and Q3 and a cycle time of 0.56 s, monitoring 16 peptides with 3 transitions each. Calibration curves were fitted with $1/x^2$ weighting, and the LLOQ was defined as the lowest level with accuracy within $\pm 20\%$ and CV $\leq 20\%$.

3. Deep and precise discovery

Discovery performance was evaluated at 500 ng K562 protein input using the 200 SPD method (Figure 2). Across four replicates, a median of 5,286 protein groups, 45,456 peptides and 53,913 precursors were identified. Quantitative precision was high, with median CVs of 4.1% at protein group and 8.3% at precursor level; 81% of protein groups were quantified below 10% CV and 96% below 20% CV. This combination of depth and precision at 200 SPD supports nomination of candidate peptides at cohort-compatible throughput.

A Identifications (n = 4)



B Quantitative precision (n = 4)

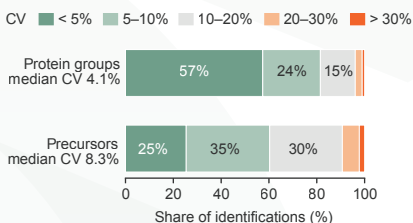


Figure 2: Discovery performance at 500 ng K562 protein input (30% loaded on Evtip, n = 4) on the SCIEX ZenoTOF 8600 with ZT Scan DIA at 200 SPD. (A) Median protein group, peptide and precursor identifications with individual replicates. (B) CV distributions at protein group and precursor levels.

4. Seamless transfer to targeted assays

A major challenge in biomarker development is converting discovery findings into quantitative assays suitable for routine use.

Peptides identified in the discovery data were transferred to a 16-peptide MRM assay on the SCIEX 7500+ without changing the sample preparation, Evtip™ loading or LC method, and analyzed across a 1–500 ng K562 dilution series.

As the Evosep Eno delivers the same gradient to both mass spectrometers, retention times were preserved between platforms, as shown for two example targets (2.9 and 3.6 min; Figure 3). At 1 ng protein input, both targets were detected on the two platforms with co-eluting transitions and clean baselines. Consistent chromatography across platforms reduces method-development effort when translating candidate biomarkers into targeted assays.

Evosep Proteomics Ecosystem

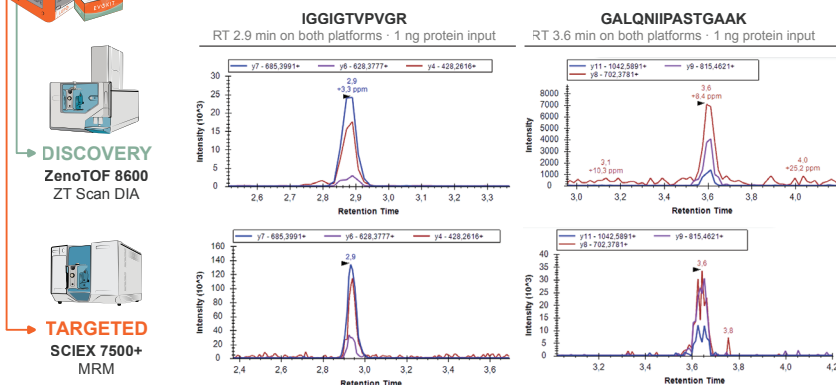


Figure 3: Extracted ion chromatograms (XICs) of two K562 peptides at 1 ng protein input by ZT Scan DIA on the SCIEX ZenoTOF 8600 (top) and MRM on the SCIEX 7500+ (bottom), using the same sample preparation and separation (Evosep Eno, 200 SPD, $n=4$).

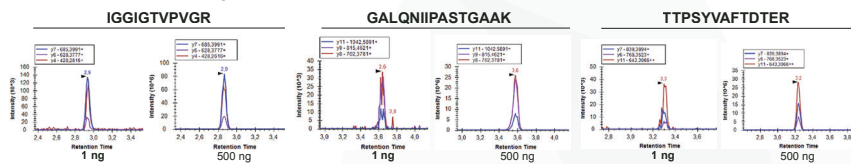
5. Quantitative accuracy

Many pharmacodynamic biomarkers are present at low abundance and may exhibit only modest treatment-induced changes, requiring sensitive and reproducible quantitative assays.

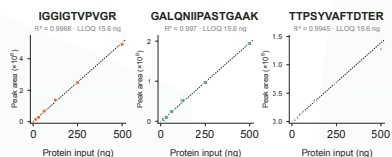
Quantitative performance of the MRM assay was evaluated across the dilution series (Figure 4). Three K562 proteotypic peptides showed linear responses with R^2 of 0.9945-0.997 and an LLOQ

of 15.6 ng protein input. Accuracy was 86–111% from 15.6 to 500 ng, within $\pm 15\%$ of nominal at every level. Below the LLOQ, peptides remained detectable with well-defined peaks down to 1 ng input. Quantification on the Evosep Eno with stable isotope-labeled standards and matrix-matched calibration in plasma has been shown previously, with 93–102% accuracy across the full sample preparation workflow [1].

A MRM traces at 1 and 500 ng protein input



B Calibration curves



C Accuracy from the LLOQ to 500 ng

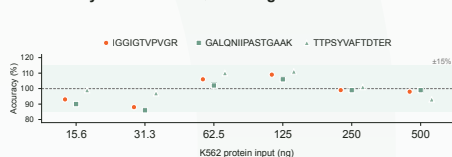


Figure 4: MRM quantification of three K562 peptides on the SCIEX 7500+ (Evosep Eno, 200 SPD, 30% of digest loaded on Evotip, $n=4$). (A) XICs at 1 and 500 ng protein input. (B) Calibration curves with R^2 and LLOQ. (C) Accuracy from the LLOQ (15.6 ng) to 500 ng with the $\pm 15\%$ range shaded.

6. Built-in quality control

Large-cohort studies require workflows that perform consistently over time. Every Evokit includes dedicated PCD samples, which pass through the full sample preparation workflow, and SSC samples, which monitor LC-MS performance only [2]. Comparing the two separates workflow variability into LC-MS and sample preparation contributions (Figure 5).

Across 1–500 ng protein input, workflow CV (PCD) remained $\leq 11.5\%$ for all three peptides and $\leq 8.2\%$ from 31.3 ng upward, with sample preparation contributing $\leq 6.4\%$. In longitudinal PD studies, where samples are often processed weeks apart, this helps distinguish analytical drift from biological change.

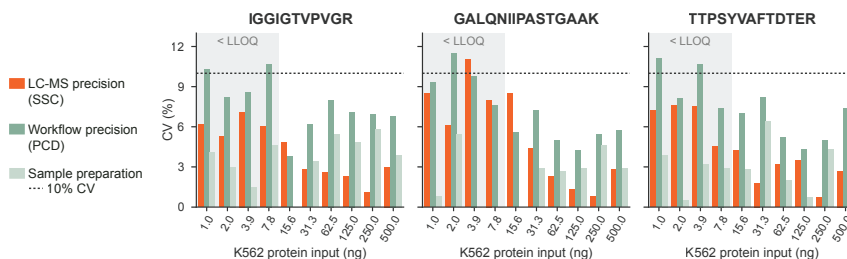


Figure 5: LC-MS precision (SSC), total precision (PCD) and sample preparation contribution for three K562 peptides across the dilution series (1–500 ng protein input). Grey area: below the LLOQ; dotted line: 10% CV. MRM on the SCIEX 7500+ at 200 SPD (n=4).

7. Conclusion

Bridging discovery proteomics and quantitative PK/PD analysis remains a key challenge in biomarker development. Here, we showcase a standardized discovery-to-targeted workflow in which sample preparation and separation remain identical across both phases. Discovery on the SCIEX ZenoTOF 8600 at 200 SPD identified a median of 5,286 protein groups with a median protein-level CV of 4.1%. Selected peptides were

transferred directly to MRM on the SCIEX 7500+ with preserved retention times, $R^2 > 0.99$ and 86–111% accuracy from 15.6 to 500 ng, while built-in PCD and SSC samples separated LC-MS from sample preparation variability.

Together with previous work on other MS platforms [1,2], these results show that the Evosep Proteomics ecosystem provides a consistent, MS-independent foundation for biomarker studies in PK/PD programs at scale.

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Evosep Lupo and Evokit are registered trademarks of Evosep in the EU.

References

- [1]. Evosep application note, AN-044F (2025) Evosep Eno - high sensitivity and quantitative accuracy for plasma proteome analysis at scale.
- [2]. Evosep application note, AN-058A (2026) Standardized sample preparation at scale with Evokits and Evosep Lupo.