

# Towards the Development of a Liquid Chromatography Free Workflow for Measurement of Clinically Important Proteins

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## Introduction

Clinical mass spectrometry (MS) for protein biomarkers has faced slow adoption due to complex workflows. The Stable Isotope Standards and Capture by Anti-Peptide Antibody (SISCAPA)-LC/MS method for thyroglobulin in autoantibody-positive patients is a rare success. However, widespread clinical adoption of mass spectrometry for measuring proteins remains stagnant as these instruments often require an expert operator and a support team to maintain their performance on a regular basis. More specifically, the need for liquid chromatography (LC) in MS workflows proves to be the main pain point to be addressed. In this study, we evaluate the robustness and transferability of an LC-free SISCAPA-trapped ion mobility spectrometry time of flight (timsTOF) method for various clinically important protein biomarkers including thyroglobulin.

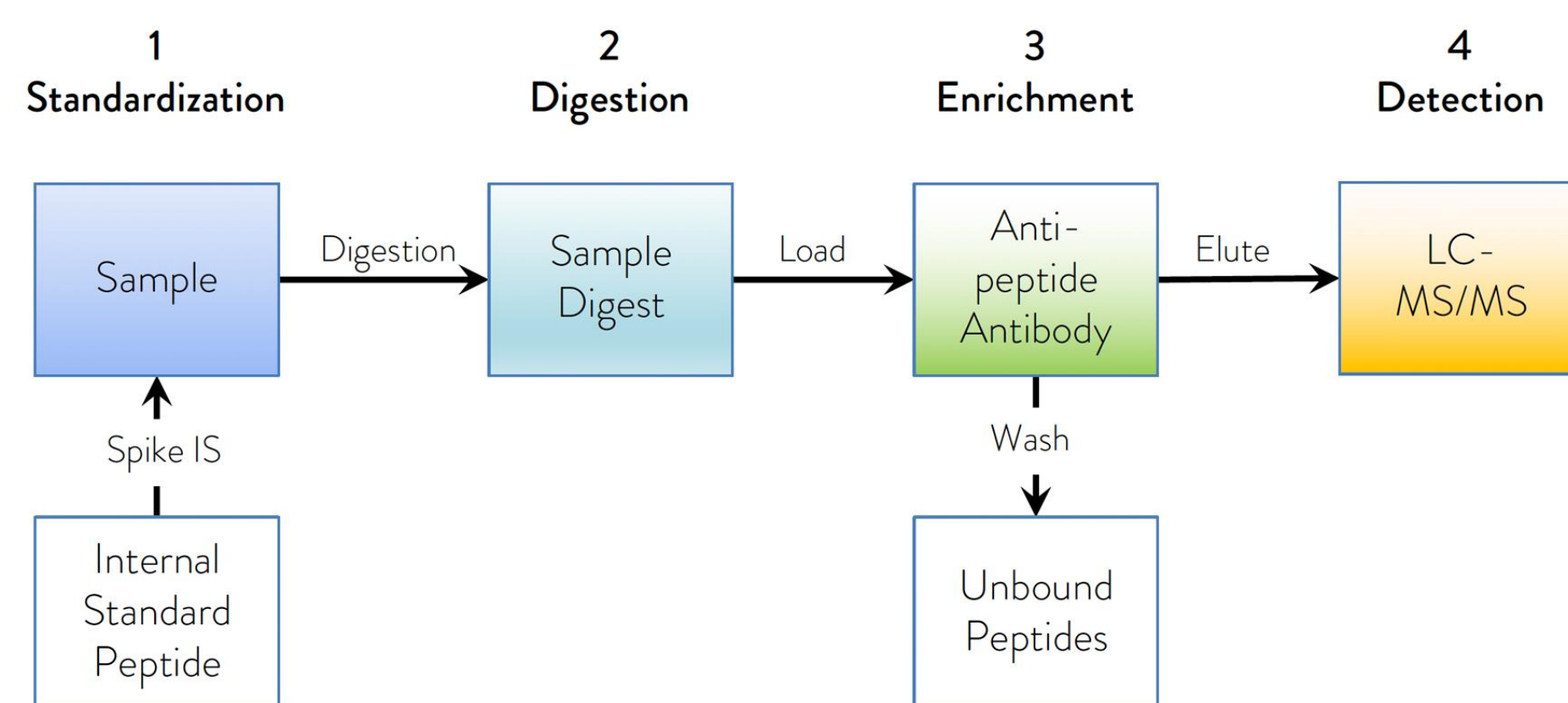


Fig 1 Stable Isotope Standards and Capture by Anti-Peptide Antibody (SISCAPA) Workflow

[1] Razavi, M., Anderson, N. L., Pope, M. E., Yip, R., & Pearson, T. W. (2016). "High precision quantification of human plasma proteins using the automated SISCAPA Immuno-MS workflow." *New Biotechnology*, 33(3), 494–502.

## Methods

Samples were digested, spiked with stable isotope-labeled peptide standards and incubated with anti-peptide antibodies conjugated to magnetic beads using the automated SISCAPA workflow (Fig 1) [1]. Following washing, captured peptides were eluted and analyzed using a timsTOF-MS platform with and without upstream LC. Data were acquired in prMSEF mode where a single quad isolation window was used to co-fragment both heavy and light peptides. LC based runs were performed with an Evosep One system (Evosep) coupled to a timsTOF HT (Bruker) with Captive Spray 2 source using throughput of 100 samples per day (SPD). For column free runs, FSPDDSAGASALLR peptide was spiked at various concentration in chicken plasma matrix and were infused directly using Elute (Bruker) into VIP-HESI source coupled to timsTOF HT at flow rate of 50  $\mu$ L/min. Data were analyzed using Skyline.

## Results

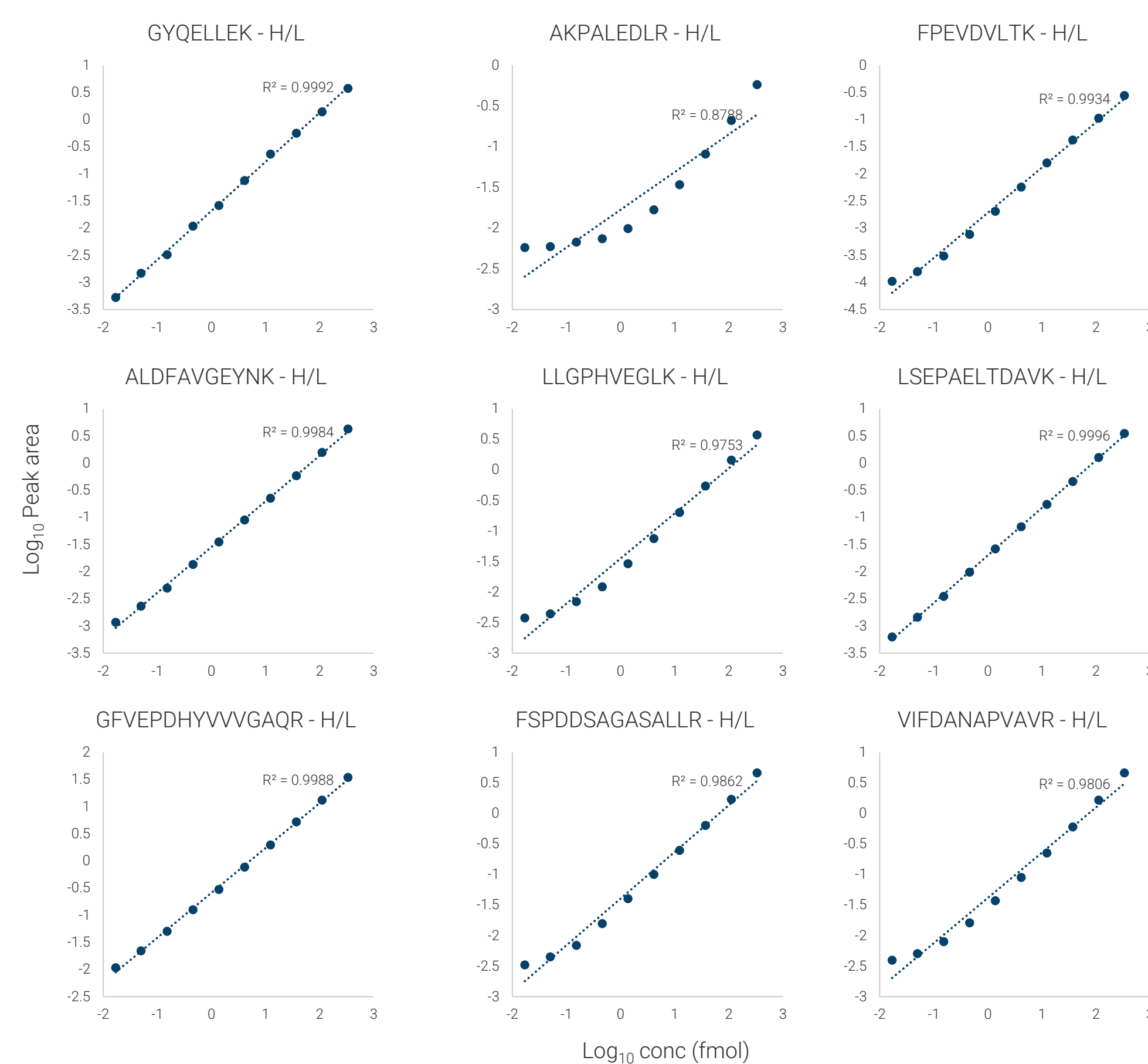


Fig. 2 Dilution curve for stable isotope-labelled peptides acquired using LC based approach on Evosep. The peptide loads are 0.017, 0.05, 0.15, 0.46, 1.37, 4.11, 12.34, 37.03, 111.11 and 333.33 fmol

Load (fmol)	GYQELLEK	AKPALEDLR	FPEVDLTK	ALDFAVGEYNK	LLGPHVEGLK
0.017	28%	4%	13%	26%	17%
0.05	29%	1%	20%	6%	6%
0.152	20%	3%	17%	7%	3%
0.457	6%	2%	9%	6%	4%
1.37	22%	2%	8%	3%	6%
4.11	2%	3%	2%	1%	3%
12.34	13%	3%	3%	2%	3%
37.03	21%	2%	2%	2%	3%
111.11	18%	2%	2%	2%	2%
333.33	5%	2%	2%	1%	1%

	LSEPAELTDAVK	GFVEPDHYVVGAAQR	FSPDDSAGASALLR	VIFDANAPVAVR
0.017	6%	7%	4%	4%
0.05	10%	15%	6%	5%
0.152	5%	9%	5%	3%
0.457	4%	4%	5%	1%
1.37	4%	1%	3%	4%
4.11	3%	3%	3%	2%
12.34	2%	3%	3%	2%
37.03	2%	4%	2%	2%
111.11	3%	1%	2%	2%
333.33	2%	3%	2%	2%

Table 1 CVs of the labelled peptides at different dilutions after normalization (Heavy/Light)

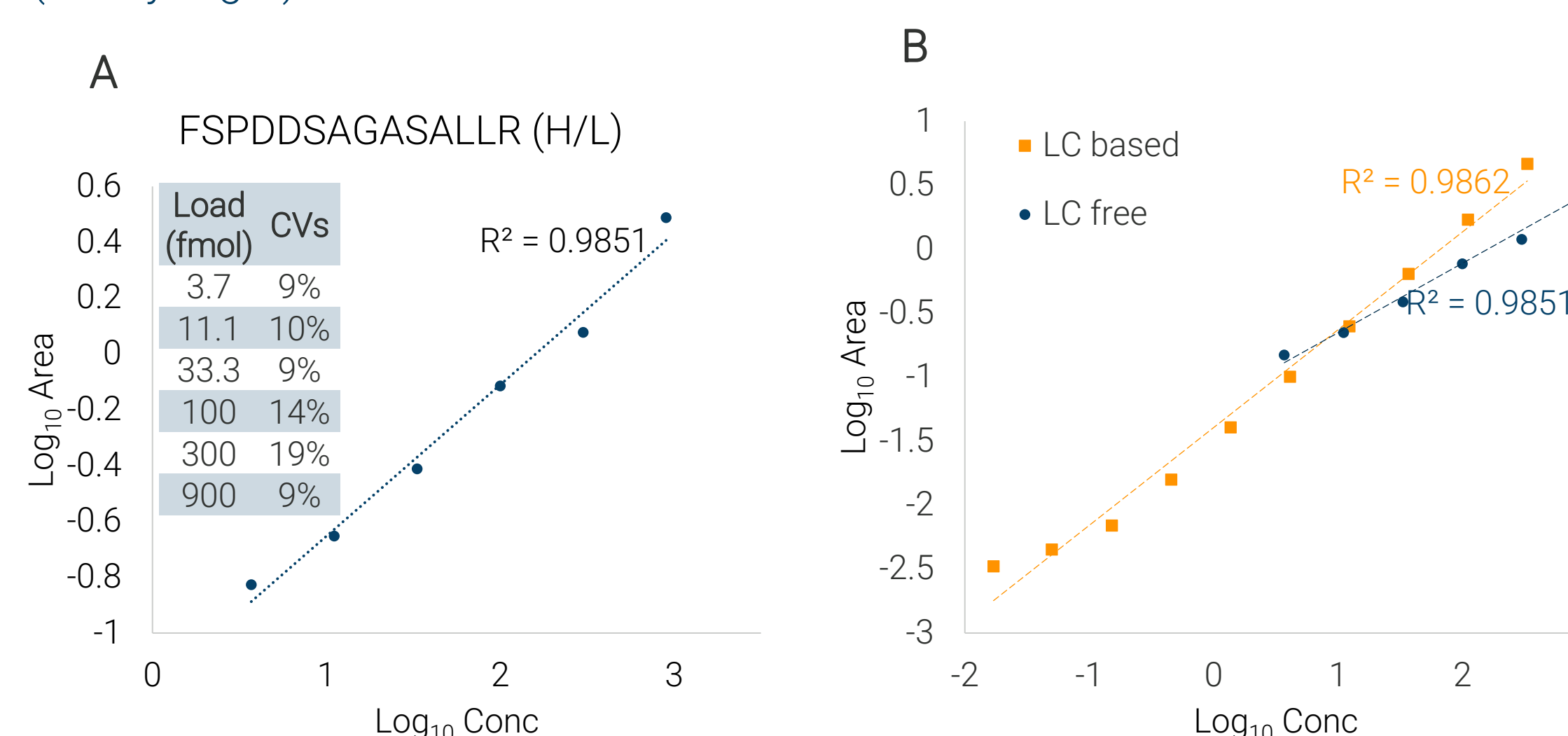


Fig. 3 A. Dilution curve for the peptide FSPDDSAGASALLR and associated CVs acquired using column free approach. The peptide loads are 3.7, 11.1, 33.3, 100, 300 and 900 fmol. B. Correlation between LC based and LC free measurements the peptide FSPDDSAGASALLR

The enrichment of target peptides by highly specific, high affinity antibodies in the SISCAPA workflow delivers a highly pure sample ready for analysis by the mass spectrometer. Consequently, we hypothesized that the use of liquid chromatography could be minimized or even eliminated during the analysis of these samples on a timsTOF instrument where the high mass resolution and TIMS separation can help compensate for the separation and chromatographic focusing proved by LC.



The initial findings indicate that there is a strong correlation between the measurements made with and without LC (Fig 3B). Our preliminary data demonstrate robust performance of both LC-based and LC-free workflows for quantifying clinically relevant protein biomarkers. For the LC-based method, excellent linearity was observed across a dynamic range from 111 fmol to 17 amol (Fig2), with CVs consistently below 10% (Table1). Similarly, the LC-free workflow achieved remarkable linearity from 900 fmol down to approximately 4 fmol, also with CVs below 20% (Fig 3A). While the LC-based workflow outperformed in the lowest concentration range, the LC-free method still demonstrated strong reproducibility and robustness at clinically significant concentration levels.

These findings highlight the potential of LC-free SISCAPA workflows to simplify sample preparation and analysis without compromising precision or accuracy. By eliminating LC, throughput increases, instrument complexity decreases, and overall workflow becomes more accessible for routine clinical applications. Furthermore, the consistent CVs across both methods indicate the reliability of SISCAPA's antibody-based enrichment for both LC and LC-free approaches.

## Summary

LC-free SISCAPA-timsTOF workflows demonstrate strong reproducibility and robustness. By eliminating LC, clinical workflows become more accessible and scalable. The technology enables high-throughput, simplified, and precise quantification of protein biomarkers, paving the way for broader clinical mass spectrometry implementation.

## Conclusion

This study demonstrates an LC-free SISCAPA-timsTOF workflow, enhancing assay robustness and simplifying protein biomarker quantification for clinical applications.

Next step is to optimize the parameters to improve the achievable sensitivity with the LC-free method as well as working to implement LC-free methods for clinically important proteins that are of higher abundance such as IGF-1

Technology

COI Statement:

RY, AH, MR and LA. are employees of SISCAPA Assay Technologies Inc. RS, MW, DH and GK are employees of Bruker Daltonics GmbH & Co. KG