

Automated quantification of liver disease associated serum proteins

using LiverEdge™ and targeted mass spectrometry

A robust, reproducible and scalable workflow for absolute proteomic quantification.

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Abstract

This study evaluates an automated sample preparation workflow for the LiverEdge™ (ProteomEdge AB, Stockholm, Sweden) targeted LC–MS/MS panel, developed for multiplex absolute quantification of liver disease-related plasma proteins using heavy isotope-labeled recombinant protein standards. Serum samples were prepared, including digestion and SPE, using a SOLO™ Automated Liquid Handler (Hudson Robotics, Springfield, NJ, USA), and subsequently analyzed on a QTRAP® 6500+ mass spectrometer (SCIEX, Framingham, MA, USA). Method repeatability, assessed across five independent replicates, demonstrated high precision, with coefficients of variation (CV) consistently remaining below 12% for the majority of peptides. By eliminating manual pipetting errors and significantly reducing hands-on time, this robust automated platform provides a standardized, scalable solution ideally suited for high-throughput clinical proteomics and large-scale cohort studies.

Introduction

Liver diseases remain a major global health challenge, highlighting the need for reliable and high-throughput analytical approaches for protein biomarker quantification. LiverEdge™ (ProteomEdge AB, Stockholm, Sweden) is a targeted LC–MS/MS panel developed for multiplex absolute quantification of liver disease-related plasma proteins using heavy isotope-labeled recombinant protein standards. The panel contains heavy-labeled Quantitative Recombinant Protein Standards (qRePS™), enabling accurate multiplex absolute quantification. The panel is provided in a convenient 96-well plate format, enabling standardized, scalable, and automation-compatible sample preparation workflows. All ProteomEdge products are delivered pre-aliquoted and dried in a standardized 96-well plate format with individual

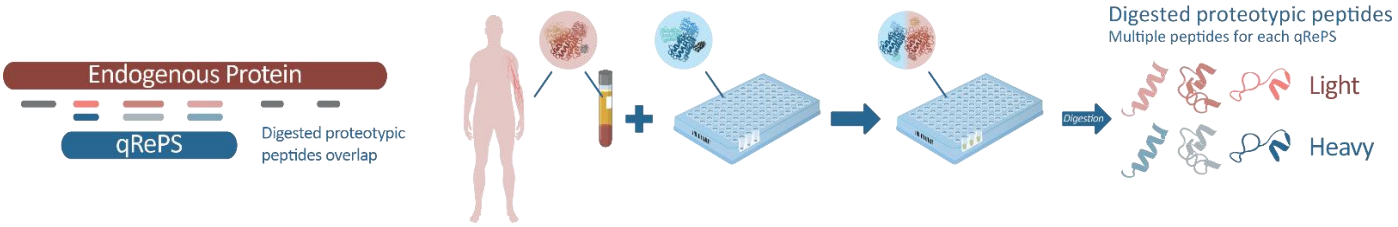


Figure 1: qRePS are recombinantly expressed protein fragments of around 100 amino acids that undergo simultaneous processing as their corresponding endogenous proteins. Samples are spiked on top of the qRePS internal standards as the first step of sample preparation providing a strategy to control analytical variability and enable multiplex absolute protein quantification.

spike-ins adjusted for digestion together with one microliter of plasma or serum and can be easily implemented in automated workflows (Figure 1).

Automation of sample preparation is essential for achieving reproducibility, robustness, and consistency in quantitative proteomics workflows. Automated liquid handling minimizes pipetting and dilution errors, reduces operator-dependent variability, and improves reproducibility across wells and plates. Furthermore, automation supports high-throughput sample processing and facilitates workflow standardization, which is particularly important for large-scale cohort studies, biomarker validation, and potential clinical applications. In this work, serum sample preparation for the LiverEdge workflow was performed using the Hudson SOLO™ Automated Liquid Handler (Hudson Robotics, Springfield, NJ, USA), allowing automated processing directly in qRePS-coated 96-well plates prior to LC–MS/MS analysis using a QTRAP® 6500+ mass spectrometer (SCIEX, Framingham, MA, USA).

Materials and Methods

Reagents and Standards

For the automated digestion procedure, the following reagents were used: 0.3% RapiGest prepared in ultrapure water, 30 mM DTT (Dithiothreitol) prepared in 1x PBS (Phosphate buffered saline), 200 mM CAA (2-Chloroacetamide) prepared in 1x PBS and trypsin (0.1 µg/µL) prepared in 1x PBS. For the solid-phase extraction (SPE) procedure, MeOH (Methanol), Solution A (0.5% acetic acid in water), and Solution B (0.5% acetic acid, 80% acetonitrile, 20% water) were used. All solvents were of LC–MS grade.

Human serum was obtained from UTAK Laboratories, Inc. (Valencia, CA, USA) and used as the biological matrix. The LiverEdge panel of qRePS targeting human liver disease-related plasma proteins was used for targeted LC–MS/MS absolute quantification.

Sample Preparation

The development of the automated sample preparation workflow was based on the ProteomEdge protocol “Digestion RapiGest” (https://proteomedge.com/wp-content/uploads/2025/12/Digestion_RapiGest_Rev4_Dec2025.pdf).

Automated sample preparation was performed using the Hudson SOLO Automated Liquid Handler according to the optimized layout (Figure 2) and sample preparation workflow (Figure 3). First, serum samples were transferred into the LiverEdge plate and subsequently subjected to denaturation, reduction, alkylation, and enzymatic digestion. Subsequently, layout of reagents and consumables were set-up according to the layout (Figure 4) and SPE was performed on the digested samples according to the protocol (Figure 5). After SPE, the samples were concentrated by evaporation using a Concentrator Plus (Eppendorf, Hamburg, Germany), operated in V-HV (vacuum – high vapor) mode at 45°C for 2 hours. The sample preparation workflow is presented in the schemes below.

The performance of both the automated digestion and SPE procedures was evaluated in terms of repeatability. For this purpose, five independent replicates were prepared and processed under identical conditions.



Figure 2: Layout of plates, tips, and heating block on the Hudson SOLO Automated Liquid Handler during the digestion procedure.

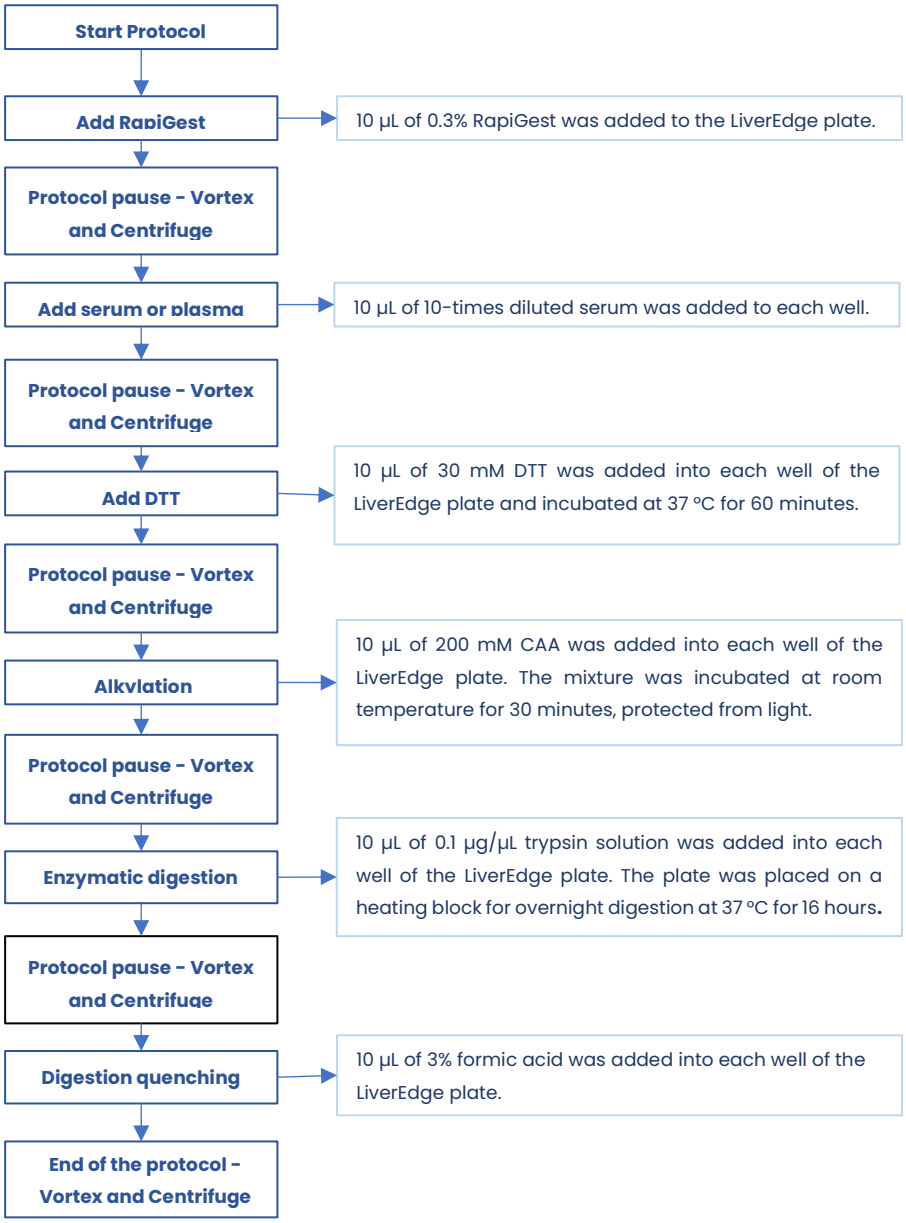


Figure 3: Overview of the automated sample preparation workflow for the Hudson SOLO Automated Liquid Handler with all the steps required during the digestion procedure.



Figure 4: Layout of plates, tips, and heating block on the Hudson SOLO Automated Liquid Handler during the solid-phase extraction procedure.

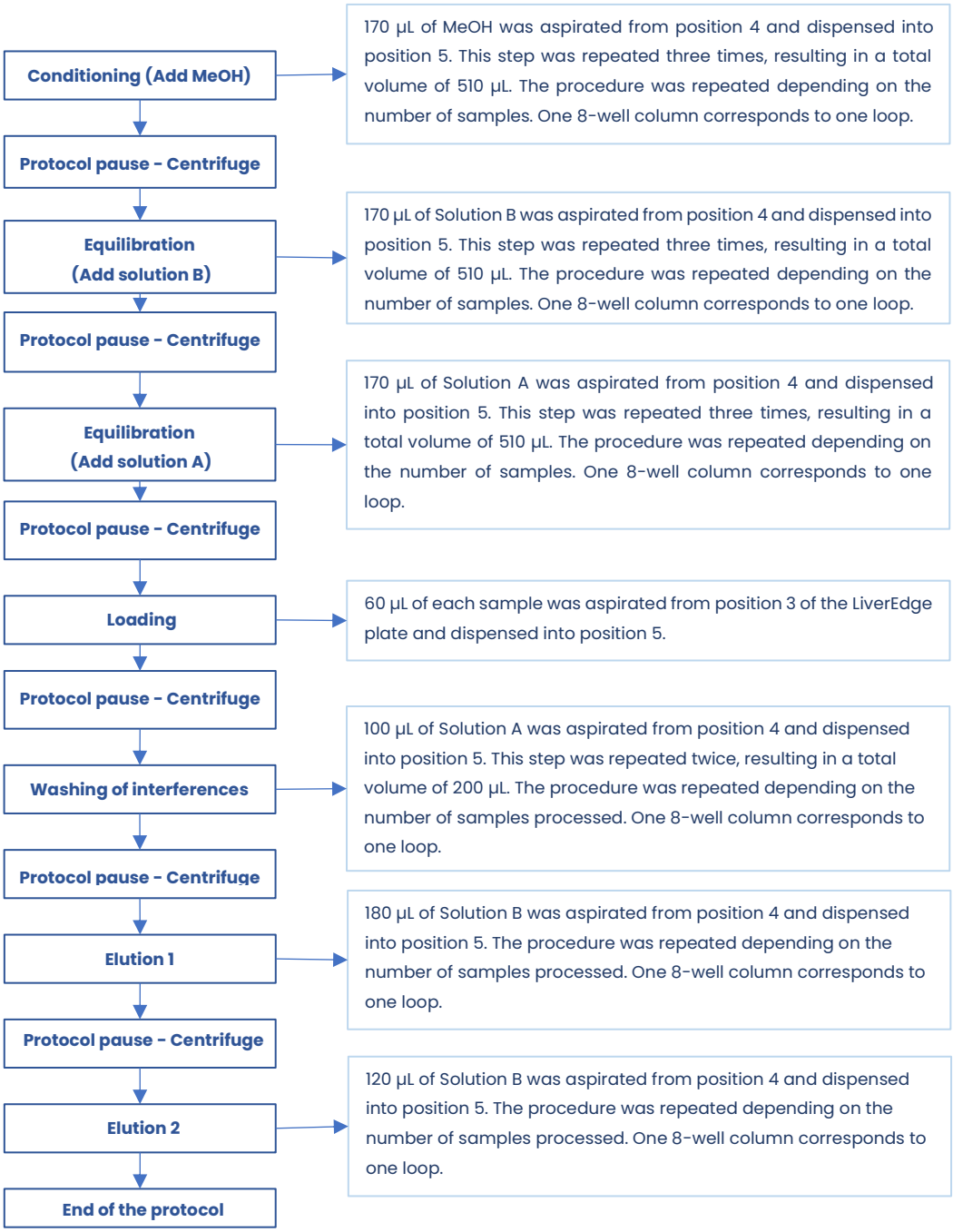


Figure 5: Overview of the automated sample preparation workflow for the Hudson SOLO Automated Liquid Handler with all the steps required during the solid-phase extraction procedure.

Chromatographic and Mass Spectrometric Conditions

Chromatographic separation was performed in reversed-phase mode using a Nexera LC-40 System (Shimadzu Corporation, Kyoto, Japan) equipped with an ACQUITY UPLC® CSH C18 Column (Waters Corporation, Milford, MA, USA) (130 Å, 1.7 µm, 2.1 × 100 mm) and an ACQUITY UPLC® CSH C18 VanGuard™ Pre-Column (Waters Corporation, Milford, MA, USA) (130 Å, 1.7 µm, 2.1 × 5 mm). A gradient elution was applied using mobile phase A consisting of 0.1% formic acid in water and mobile phase B consisting of 0.1% formic acid in acetonitrile.

Mass spectrometric analysis was performed using the SCIEX QTRAP® 6500+ MS/MS System operating in Multiple Reaction Monitoring (MRM) mode with electrospray ionization (ESI). Detailed chromatographic conditions, gradient parameters, mass spectrometer settings, and all MRM transitions are provided in the *Supporting Materials*.

Results

Data processing method was developed using SCIEX OS Software with the Analytics Module and MQ4 Integration Algorithm (SCIEX, Framingham, MA, USA). To assess repeatability, the coefficient of variation (CV) was calculated based on quantitative results for each peptide. Due to the large number of results, one representative peptide per protein was presented. The CV across five independent replicates did not exceed 12% with median CV of 5.7% (Table 1), confirming high method repeatability, indicating that the automated sample preparation, MS analysis and absolute quantification can be reliably applied under laboratory conditions and across wide dynamic range of serum protein concentrations (Figure 6).

Table 1: Absolute serum protein concentrations for each biological replicate and their corresponding coefficients of variation (CV).

	Serum Concentration [pmol/µl]							
Protein_Peptide	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5	Average	SD	CV [%]
C3_ILLQGTPVAQMTEDAVDAER	11.1900	10.3223	10.1873	9.8004	9.6774	10.2355	0.5962	5.8
SERPINC1_DIPMNPMCIYR	4.8914	4.5749	4.3548	4.4185	4.4087	4.5297	0.2182	4.8
VTN_GQYCYELDEK	3.8032	3.5959	3.5078	3.4088	3.4054	3.5442	0.1648	4.7
CFH_SIDVACHPGYALPK	2.2662	2.1635	2.0643	2.1434	2.0566	2.1388	0.0854	4.0
F2_LAVTTHGLPCLAWASAQAK	2.0023	1.8855	1.8001	1.7680	1.7578	1.8427	0.1024	5.6
RBP4_QEELCLAR	1.9310	1.8169	1.7923	1.8430	1.7930	1.8352	0.0574	3.1
ITIH4_SPEQQETVLDGNLIIR	1.7712	1.6537	1.5359	1.5843	1.5332	1.6157	0.0997	6.2
APOA4_ALVQQMEQLR	1.3587	1.2865	1.2377	1.2556	1.2467	1.2770	0.0492	3.9
APOE_EQVAEVR	1.1187	1.0432	1.0625	1.0192	0.9718	1.0431	0.0542	5.2
SERPINF2_LCQDLGPGAFR	0.8941	0.8182	0.7783	0.7715	0.7733	0.8071	0.0523	6.5
APOM_FLLYNR	0.8425	0.7890	0.7897	0.7599	0.7579	0.7878	0.0342	4.3
AFM_FTFEYSR	0.6625	0.5982	0.5757	0.5952	0.5855	0.6034	0.0342	5.7
C7_GGGAGFISGLSYLELDNPAGNKR	0.3272	0.2864	0.2986	0.2955	0.3111	0.3037	0.0158	5.2
SERPINF1_LQSLFDSPDFSK	0.2987	0.2875	0.2724	0.2737	0.2697	0.2804	0.0123	4.4
ORM1_EQLGEFYEALDCLR	0.2645	0.2437	0.1982	0.2615	0.2427	0.2421	0.0265	10.9
FBLN1_TGYYFDGISR	0.2371	0.2323	0.2263	0.1981	0.2096	0.2207	0.0164	7.4
SELENOP_VSLATVDK	0.2015	0.1838	0.1751	0.1894	0.1729	0.1846	0.0116	6.3
ATRN_SEAACLAAGPGIR	0.1858	0.1624	0.1719	0.1718	0.1670	0.1718	0.0088	5.1
THBS4_AFAGPSQKPETIELR	0.1386	0.1151	0.1087	0.1076	0.1221	0.1184	0.0127	10.7
LGALS3BP_SQLVYQSR	0.1108	0.1002	0.0959	0.1071	0.0962	0.1021	0.0066	6.5
CNDP1_SVVLIPLGAVDDGEHSQNEK	0.0783	0.0748	0.0660	0.0669	0.0707	0.0714	0.0052	7.3
LBP_LAEGFPLPLLK	0.0685	0.0575	0.0519	0.0578	0.0544	0.0580	0.0064	11.0
FCN2_LGEFWLGNDNIHALTAQGTSELR	0.0410	0.0455	0.0455	0.0423	0.0438	0.0436	0.0020	4.6
TGFBI_EGVYTVFAPTNEAFR	0.0303	0.0253	0.0272	0.0274	0.0270	0.0274	0.0018	6.6
PIGR_AFVNCDENSR	0.0291	0.0269	0.0251	0.0262	0.0211	0.0257	0.0029	11.5
CLEC3B_SRLDTLAQEVALLK	0.0126	0.0131	0.0129	0.0123	0.0128	0.0127	0.0003	2.3
ALDOB_LDQGGAPLAGTNK	0.0100	0.0084	0.0103	0.0085	0.0111	0.0097	0.0011	11.9
FN1_GLAFTDVDVDSIK	0.0026	0.0021	0.0020	0.0025	0.0022	0.0023	0.0002	11.0

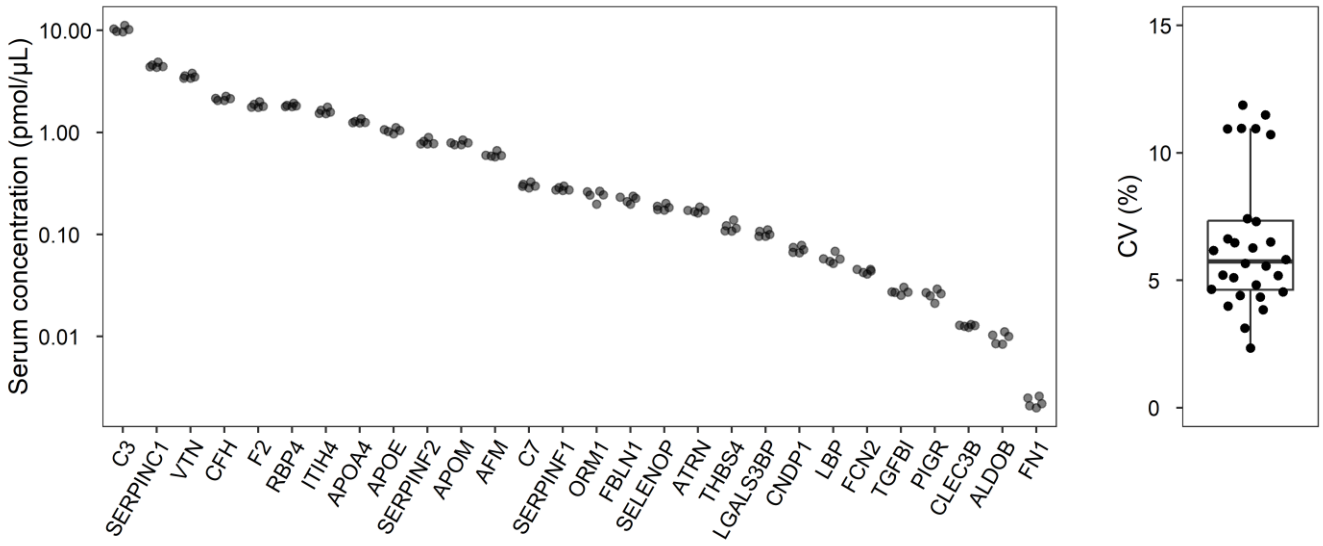


Figure 6: Absolute serum concentrations of the 28 different liver markers quantified in 5 biological replicates with the CVs distribution and median CV of 5.7%.

For the representative peptides shown in Table 1, CV values remained below 12%, confirming good method repeatability and demonstrating that the automated sample preparation workflow can be reliably applied under laboratory conditions. The only exception was QSOX1_DFNIPGFPTVR for which a CV value of 16.16% was obtained. However, considering the low signal intensity observed for this peptide, the result can still be regarded as acceptably repeatable. For ADIPOQ, ANPEP, APOF, COL1A1, CST3, DPP4, PEPD, PROC and SPP1, only heavy-labeled peptides were detected therefore, quantitative results for endogenous peptides could not be calculated and are not included in the table. Nevertheless, successful detection of the heavy peptide standards for these proteins confirms correct method performance and proper execution of the automated workflow.

Good chromatographic separation and highly stable retention times were achieved across all five biological replicates, demonstrating excellent reproducibility of the LC–MS/MS method under analytical flow conditions (Figure 7). Overlay of the total ion chromatograms (TICs) showed consistent peptide elution profiles with minimal retention time drift between runs, indicating robust chromatographic performance and stable instrument operation throughout the analysis. The combination of automated sample preparation, reproducible chromatographic separation, and targeted MRM detection enabled reliable multiplex quantification of liver disease-associated proteins over a broad dynamic concentration range. The robustness and reproducibility of the workflow support its suitability for routine high-throughput applications, enabling processing and analysis of up to 96 samples per day in clinical proteomics and large-scale cohort studies.

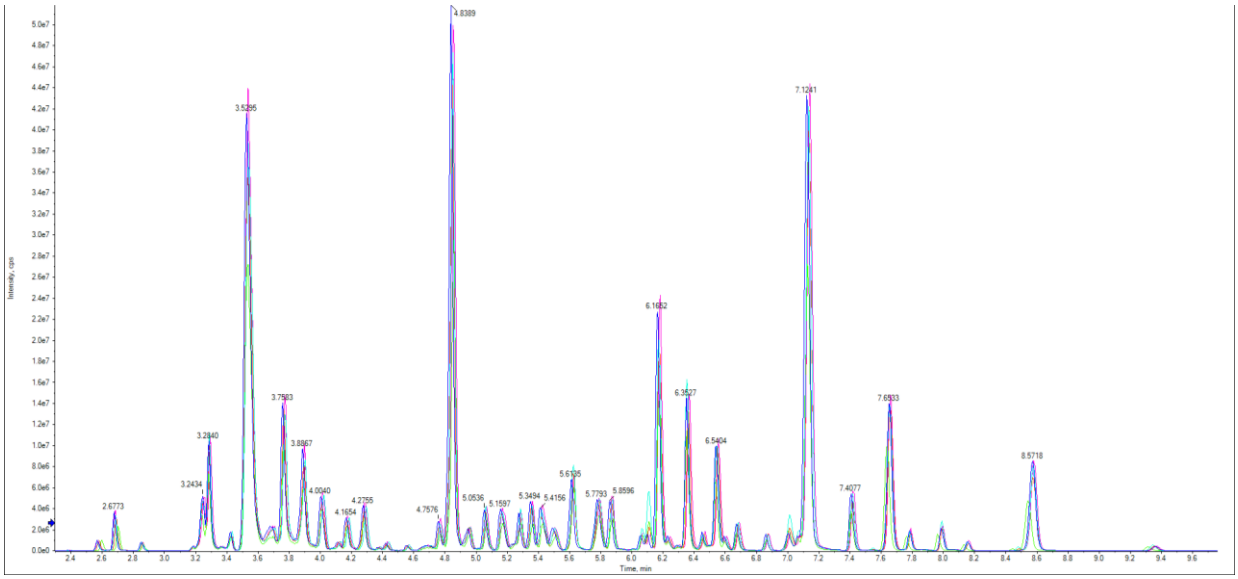


Figure 7: Overlay of total ion chromatograms (TIC) for five independent replicates.

Conclusion

- The automated sample preparation in combination with robust MS readout and implementation of internal standards early in the workflow showed high repeatability, as demonstrated by five independent replicates with a maximum CV not exceeding 15%, confirming that the method is robust and reliable for quantitative applications.
- The use of the Hudson SOLO Automated Liquid Handler offers significant advantages over manual sample processing, including reduced pipetting and dilution errors, minimized operator-dependent variability, and improved consistency both within and between plates. In addition, automation substantially decreases hands-on time improving laboratory efficiency and enabling more effective allocation of resources.
- The automated workflow together with ready-to-use panels supports high-throughput sample processing and is therefore well suited for large-scale cohort studies, enabling simultaneous preparation of multiple samples with high consistency.
- Standardization and integrated quality control throughout the complete workflow enhance method transferability, reproducibility, and scalability, which are critical for clinical and proteomics applications. Reproducible workflows implementing panels of internal protein standards, such as LiverEdge, are key enablers of reproducible and harmonized sample preparation, ensuring robust and reliable quantitative results.

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