SHIMADZU
Excellence in Science

PO-CON1486E

Online affinity and digestion: A flexible and robust tool for the characterization and quantification of proteins

ASMS 2014 WP161

David Colquhoun[1]; Mohamed Nazim Boutaghou[1]; Rachel Lieberman[1]; Brian Feild[1]; Kevin W. Meyer[2]; Scott Kuzdzal[1]
[1]Shimadzu Scientific Instruments, Columbia, MD;
[2]Perfinity Biosciences, West Lafayette, IN

Online affinity and digestion: A flexible and robust tool for the characterization and quantification of proteins

Introduction

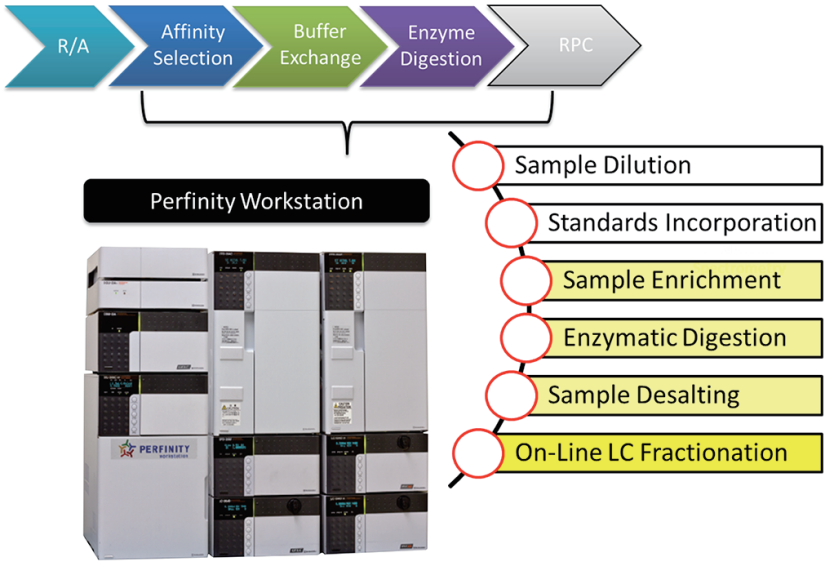
Traditional, manual proteolytic digest and immuno-affinity sample preparation techniques have been plagued by their lack of qualitative and quantitative reproducibility. This has contributed in part to the lack of implementation of protein biomarkers in quantitative MS-based assays. Online automation of digestion can reduce variability, user error, and improve quantitative results. If coupled with online affinity capture using immobilized antibodies or affinity columns, a unique front-to-end solution can be utilized that allows reproducible sample analysis without time-consuming manual protocols. This work demonstrates the flexibility and utility of a completely online, automated solution for LC-MS/MS sample preparation and peptide analysis using both data dependent and targeted workflows.

Methods

- Online affinity capture was performed using an immobilized anti-Hb column and eluted directly onto an immobilized enzyme reactor (IMER) and subsequently to reversed phase separation.
- Using variables including time, washing stringency and temperature, we optimized each component of the affinity and online digestion process.
- Protein sequence coverage was analyzed using data dependent acquisition on a Shimadzu LCMS-IT-TOF.
- Peptides were selected and optimized using Skyline. Peptide MRM transitions were monitored using the same Perfinity Workstation coupled to an ultra-fast LCMS-8050.

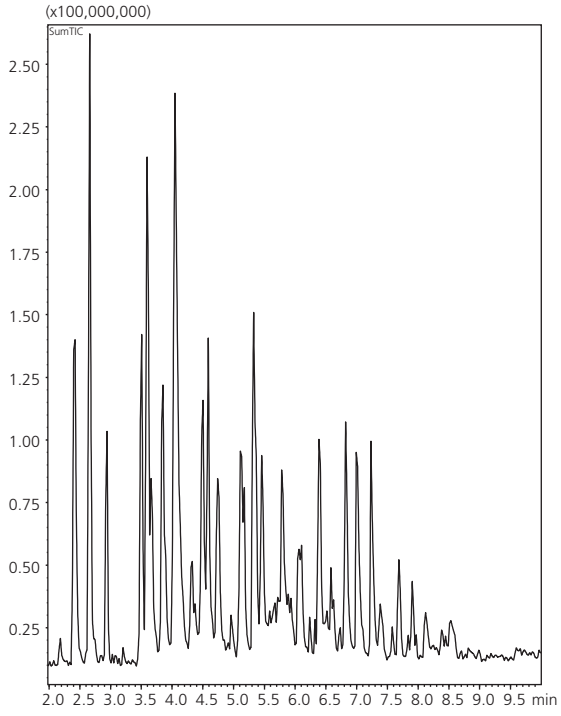
Workflow

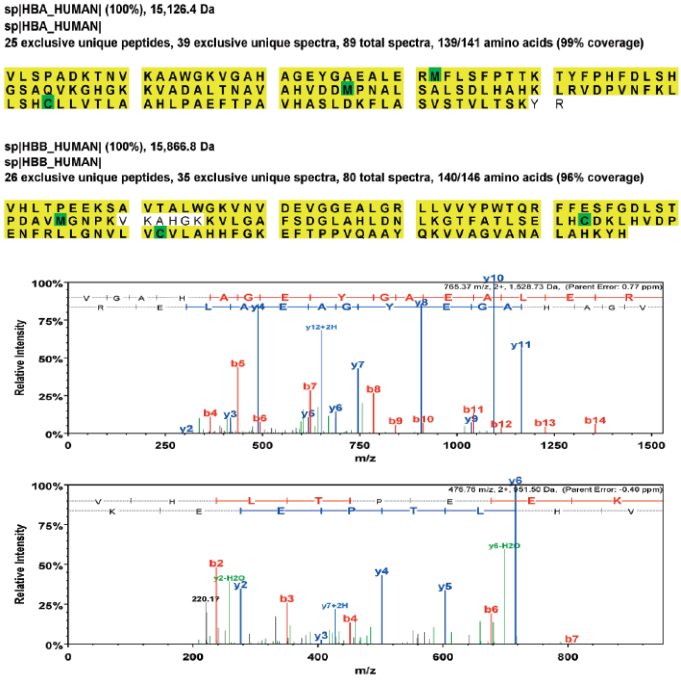
- Import Skyline predicted transitions directly into LabSolutions
- Identify the most favorable transitions and optimize source and CID conditions
- Output data files directly into Skyline and LabSolutions



Online affinity and digestion: A flexible and robust tool for the characterization and quantification of proteins

Rapid Digestion: Hemoglobin





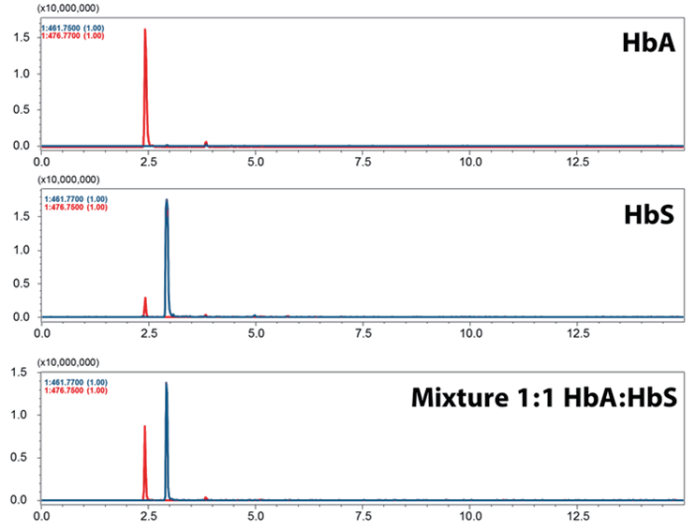


Figure 1. Data dependent discovery (DDA) of protein isoforms. Digestion using the Perfinity Workstation (PWS) was optimized for hemoglobin variants (4 minute digestion at 40˚C) and mixtures were analyzed using the LCMS IT-TOF with a top 3 approach on a 15-minute gradient at 0.5 mL/min. Coverage at 5 % protein FDR was approaching 100 %, and isoform peptides were identified.

Online affinity and digestion: A flexible and robust tool for the characterization and quantification of proteins

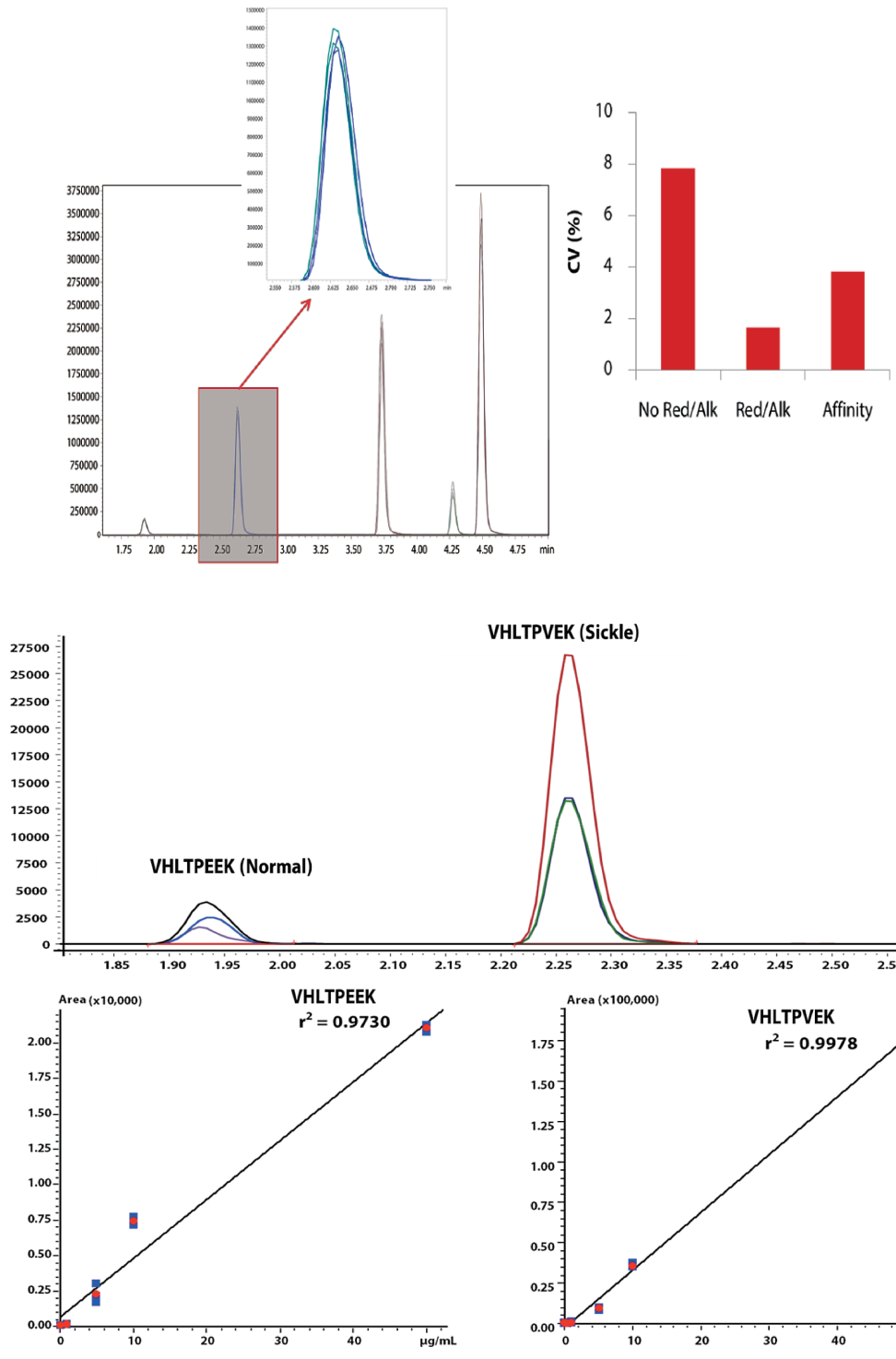


Figure 2. Reproducible, accurate quantitative analysis of protein isoforms. Protein isoforms identified via DDA were analyzed using the PWS coupled to an LCMS-8050 using the digestion and affinity parameters optimized in previous experiments. The sickle and wild type peptides were resolved and quantified using a 5 minute gradient at 0.5 mL/min. CVs for the acquisition were below 8 % for all experiments.

Digestion and Immuno-MRM: IgG

- Immuno-MRM incorporates online affinity capture, digestion and LC-MS/MS
- Automated using Perfinity Workstation with full method completed in under 1 hour
- Identified constant and variable region peptides and optimized using Skyline

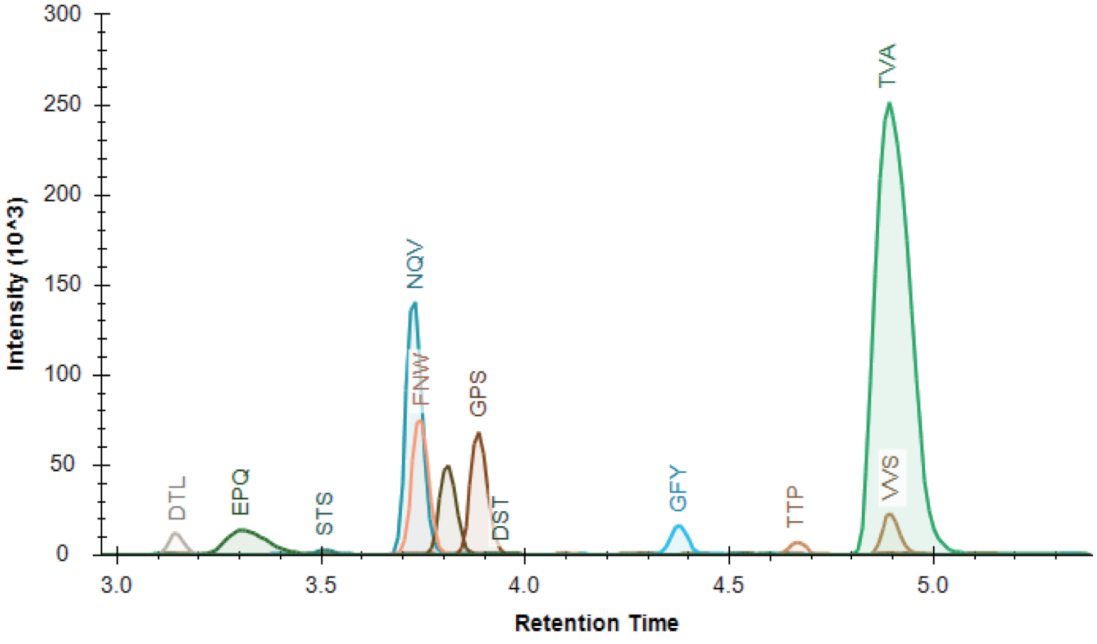
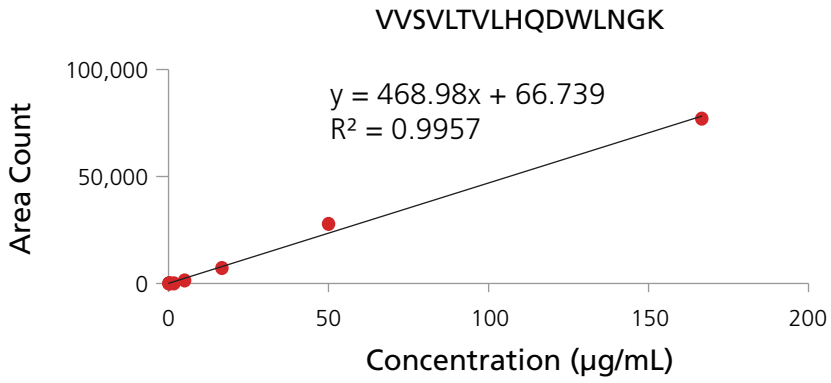
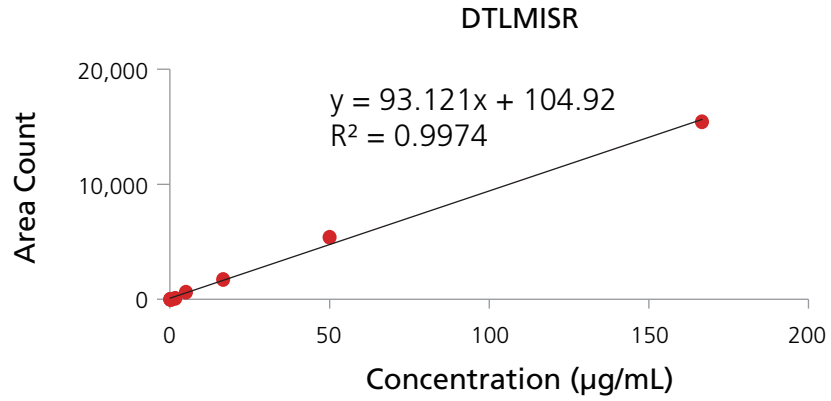
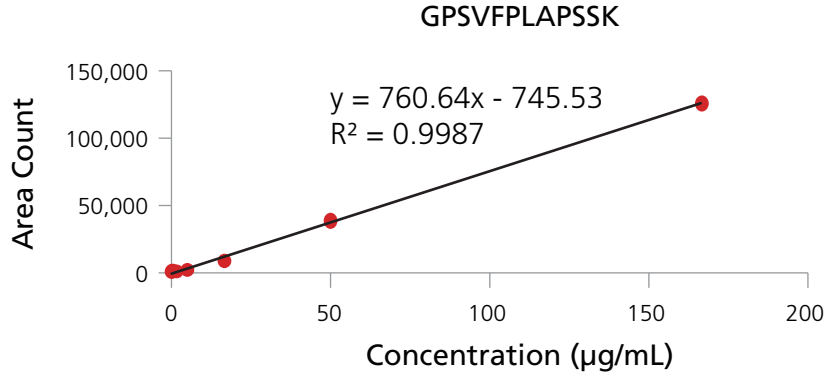


Figure 3. Rapid immuno-MRM of human IgG. Samples were affinity captured using a Protein G column, digested and analyzed using a Perfinity Workstation coupled to an LCMS-8050 triple quadrupole. Target peptides and transitions were selected using Skyline; source and CID conditions were optimized manually. A standard curve was generated and 12 peptides from the constant and variable regions of IgG were monitored.







Summary

- The Perfinity Workstation facilitated rapid method optimization for affinity enrichment and digestion
- Data-dependent analysis resulted in high (>90 %) sequence coverage for target proteins
- The same Workstation was coupled to an LCMS-IT-TOF and then an LCMS-8050, allowing seamless transition from discovery to targeted analysis
- The Perfinity Workstation automated affinity capture, digestion and separation resulting in rapid analysis times (20 - 60 min) , low CVs (<8%) and sensitive detection.

Online affinity and digestion: A flexible and robust tool for the characterization and quantification of proteins

Acknowledgements

The authors would like to acknowledge the assistance of the Applications Laboratory team at Shimadzu Scientific Instruments with experimental design and execution. We would also like to thank John Schiel (NIST) for the gift of Candidate NIST RM 8670 monoclonal antibody lot #3F1b.

First Edition: June, 2014

SHIMADZU

Shimadzu Corporation

www.shimadzu.com/an/