Faster protein digestion without loss of fidelity: rapid desalting, optimized buffers and heat-stabilized immobilized trypsin

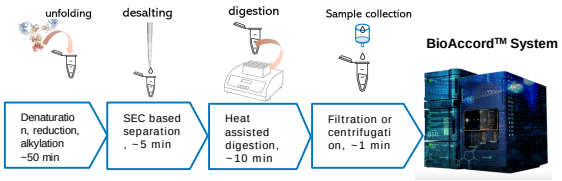
Wenjing Li; Beatrice Muriithi; Oksana D. Tchoul; Anna Boardman; Donna Osterman; Mingcheng Xu; Nicholas Zampa; Matthew Lauber
Waters Corporation, 34 Maple St, Milford MA 01757

Waters
THE SCIENCE OF WHAT'S POSSIBLE.™

Introduction

Peptide mapping is a powerful and versatile analytical approach to investigate stability and biotransformation throughout the lifecycle of any protein therapeutic. However, the sample preparation usually involves tedious, time-consuming steps that need to be optimized and later validated. This also poses a tremendous challenge to inter- or intra-lab reproducibility. To date, most protocols include a desalting step that introduces highly variable protein recoveries and a digestion step that can take over an hour, and sometimes even an overnight incubation, to complete. Here, we present a protocol that features a rapid desalting technique providing uniform protein recoveries (>85%) and a heat-stabilized immobilized trypsin to achieve complete, high fidelity IgG digestion in 10 min.

Experimental



NIST mAb was used to optimize individual facets of protein digestion sample preparations. In an example 30 µL of 10 mg/mL protein was denatured and alkylated. Desalting tips were equilibrated with 50 mM Tris-HCl buffer (pH 7.5) before 100 µL of denatured protein was added to the top of the tip and eluted with 150 µL of 50 mM Tris. 35 µL of desalted protein and 165 µL of optimal digestion buffer was added to each vial containing immobilized trypsin slurry, and the mixture was incubated on a thermal mixer at 70 °C for 10 min. 150 µL of samples were filtered and digest were acidified with formic acid before being subjected to LC-MS analysis on a BioAccord system (Waters).

Results

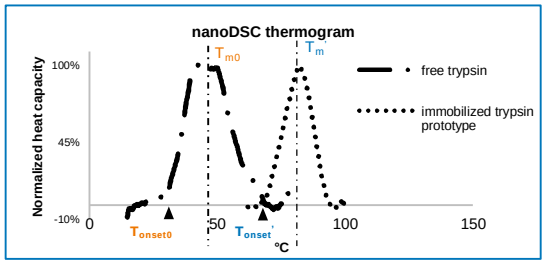


Figure 1. NanoDSC thermograms of free trypsin and one optimal immobilized enzyme prototype.
T_m – temperature where half of the protein is unfolded;
T_{onset} - temperature where protein starts to unfold.

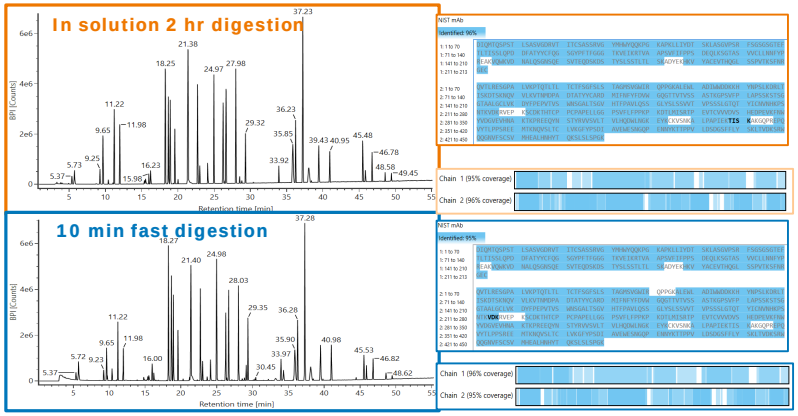
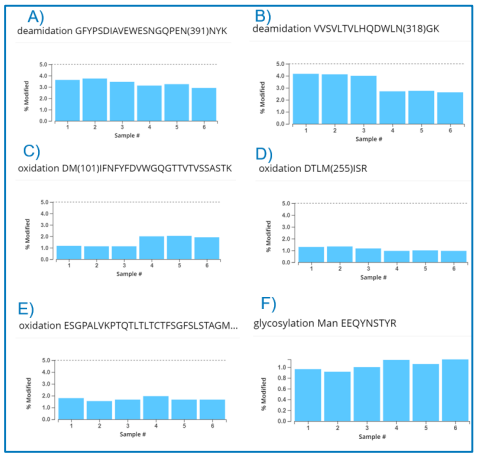


Figure 2. The peptide mapping results comparing the 2 hr trypsin digestion workflow vs. the 10 min fast digestion workflow. Left panel: LC-MS chromatogram; Right panel: sequence coverage of light chain and heavy chain respectively.

Figure 3. The CQA peptide monitored through the Peptide MAM app in waters_connect ™ software.
Sample #1-3: replicates of 2 hr trypsin digestion workflow;
Sample#4-6: replicates of 10 min fast digestion workflow
A-B) deamidation% for 2 asparagine-containing peptides; C-E) oxidation% of 3 methionine-containing peptides; F) glycosylation% of the mannose-5 glycoform for peptide EEQYNSTYR



Summary

Based on these new developments, a one-hour sample preparation process for peptide mapping has been devised that shows results comparable to lengthier, gold standard procedures. NIST mAb has been used for method development and to confirm high-fidelity digestion results. From the new protocol, total percent area from miss-cleavage peptides has been confirmed to be less than 15%; sequence coverage has been found to be above 95%; and modification of oxidation- or deamidation-prone peptides was controlled to be within 1% to in solution 2 hr tryspin digestion results. The streamlined data acquisition and analysis supported by Peptide MAM app as part of waters_connect software enables further simplification of the full workflow for MAM users.