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Flexible Automated Sample Preparation Workflows: Modified Automated Systems for Specific Immuno-MS and MS Workflows

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David Colquhoun[1], M. Nazim Boutaghou[1], Nishi Rochelle[1], Brett Nole[2], Laurie Parker[2], Kevin Meyer[3], Scott Kuzdzal[1], and Brian Feild[1].
[1]Shimadzu Scientific Instruments, Columbia, MD;
[2]University of Minnesota, Minneapolis, MN
[3]Perfinity Biosciences, West Lafayette, IN

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Introduction

The automation of sample preparation has become an increasingly important component for reproducible and operator-independent experiments. The Perfinity Workstation has provided researchers established applications in affinity capture and digestion for targeted proteomic workflows, utilizing specific affinity, IMER and reversed phase components coupled directly to a mass spectrometer. This approach offers the benefits of both specificity and resolution. However, utilizing alternative existing chemistries and multi-dimensional approaches, it is possible to reconfigure systems with a great deal of flexibility (e.g, pepsin digestion, HILIC, fraction collectors (FRC) or intact protein separation). The automated systems can then be utilized for a host of applications including targeting post-translationally modified proteins, non-tryptic peptides, and intact proteins. This work outlines novel strategies that are being utilized for automated online and offline sample preparation to achieve these specific goals.

Methods

Protein standards included BSA, IgG and insulin (10 µg/mL). Methods, valve and column positions were modified as needed.

To rapidly assess the digestion efficiency (over 1-8 min), the RPC column was removed and peptides eluted in acetonitrile to a fraction collector (0.5 – 1.0 mL). Eluents were dried in a SpeedVac for 30 -45 minutes at 50 °C. Samples were spotted onto a stainless steel target place with 10 mg/mL CHCA and analyzed by using an Axima Performance MALDI-TOF MS in reflectron mode with a PE of 3000 and a mass range of *m/z* 500 – 4,000. Calibration was carried out using a peptide calibration mix. Spectra were processed for baseline correction and smoothing using standard parameters.

To assess alternate digestion, a custom Perfinity IMER pepsin column was used (Perfinity Biosciences, West Lafayette, IN). Buffer and digestion conditions were optimized by monitoring the digestion of insulin by UV over 1-8 minutes. Initial use of 40 mM HCl resulted in the leaching of metals from the sinkers, so alternative buffers were surveyed. The standard workstation configuration was used with the pepsin column replacing the trypsin column. Wash buffer was 5 % ACN and 5 % IPA in 20 % acetic acid. Reversed phase separation was carried out using a gradient of 5 – 50 % acetonitrile over 5 minutes, with detection at 214 nm.

For additional workflows, specified customizations were utilized and the Perfinity Workstation was combined with the relevant instrumentation (e.g., LCMS-8050).

Rapid digest optimization using MALDI-TOF MS

Figure 1. Rapid digestion coupled with MALDI analysis. Digestion was carried out in 1-8 minutes using a Perfinity Workstation. The resulting digest was trapped and eluted in a single fraction, dried and spotted for MALDI-TOF MS analysis. MALDI spectra revealed that increased digestion time resulted in improved digestion, with optimal signal observed at 4 minutes. The entire method was completed in less than 2 hours.

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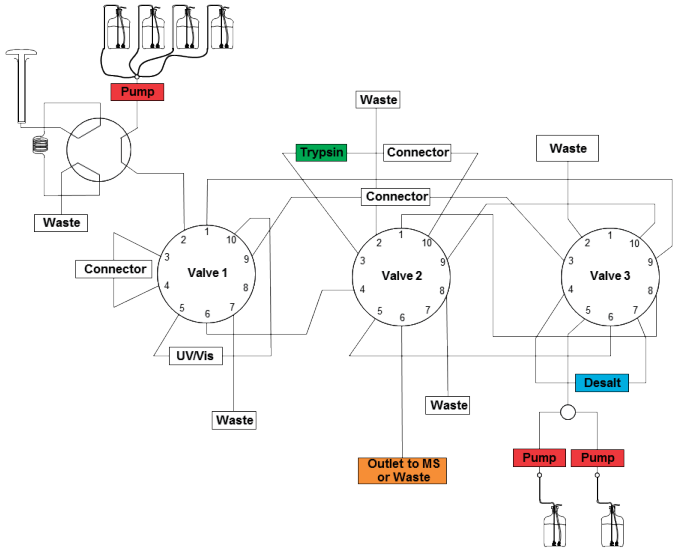


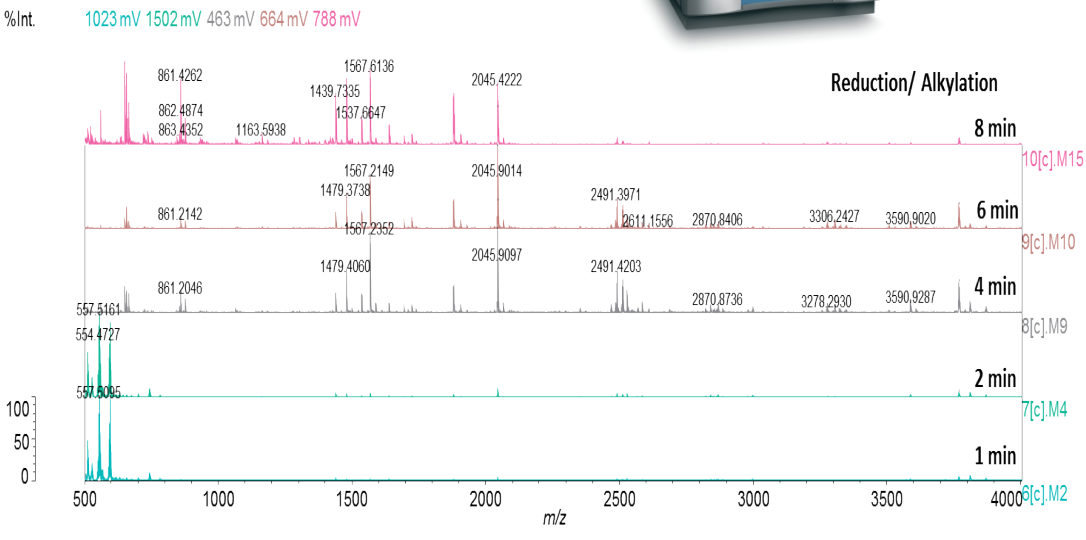


Fig.1 Rapid digestion coupled with MALDI analysis.

Non-tryptic Digestions with a UV workflow

Figure 2. Digestion using a non-tryptic immobilized enzyme column. The column was prepared using immobilized pepsin. The digestion buffers and conditions are shown in the key above. The configuration used was a standard digestion to UV method, with modified buffers (see key). Insulin digestion was optimized over 1 – 8 minutes (top right). Following optimization, the reproducibility of digest was measured using consecutive injections (bottom right panel).

Column	: Custom Perfinity Immobilized Pepsin column
Buffers	: Digestion buffer (B) : 100 mM sodium acetate pH 4.5 in water
	Wash buffer (D) : 5 % IPA/ 5 % ACN/ 20 % acetic acid in water
Samples	: 1.0 mg/mL insulin or 1.0 mg/mL hemoglobin
	In 90 % Buffer B/ 10 % (50:50 Tris: 6 M Guanidine HCl)

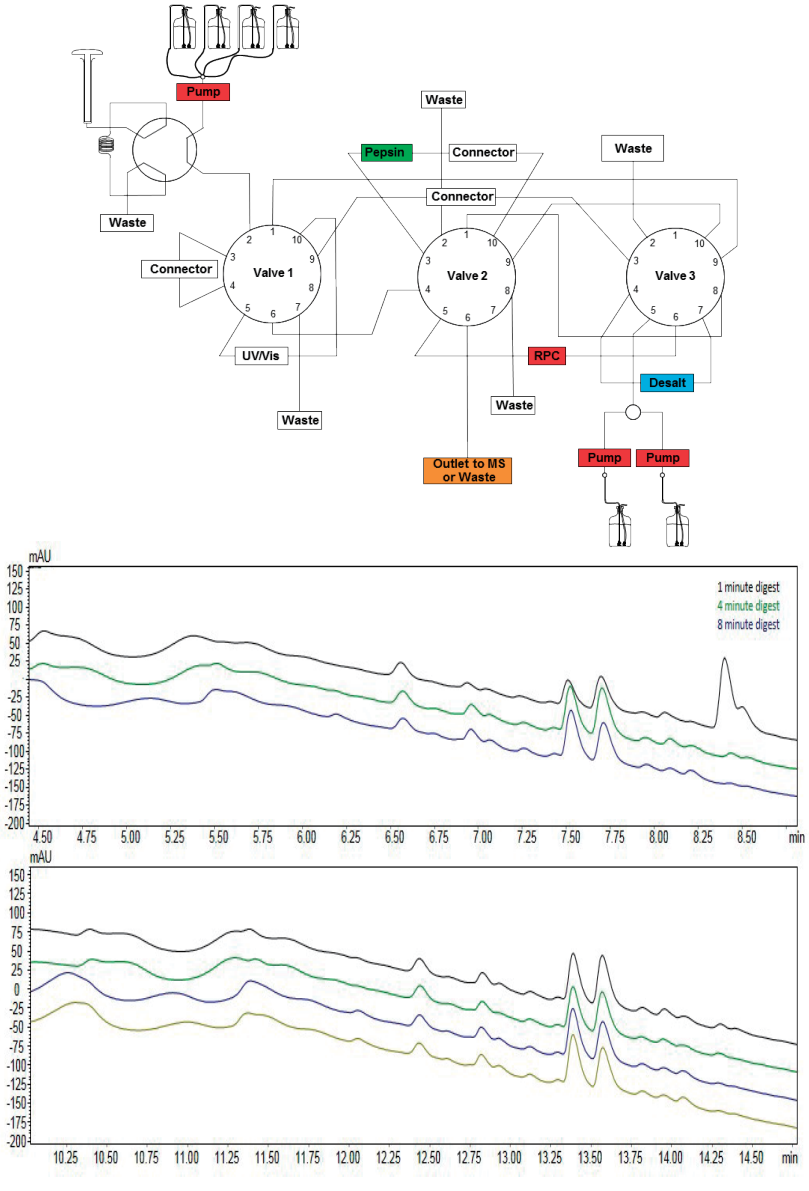
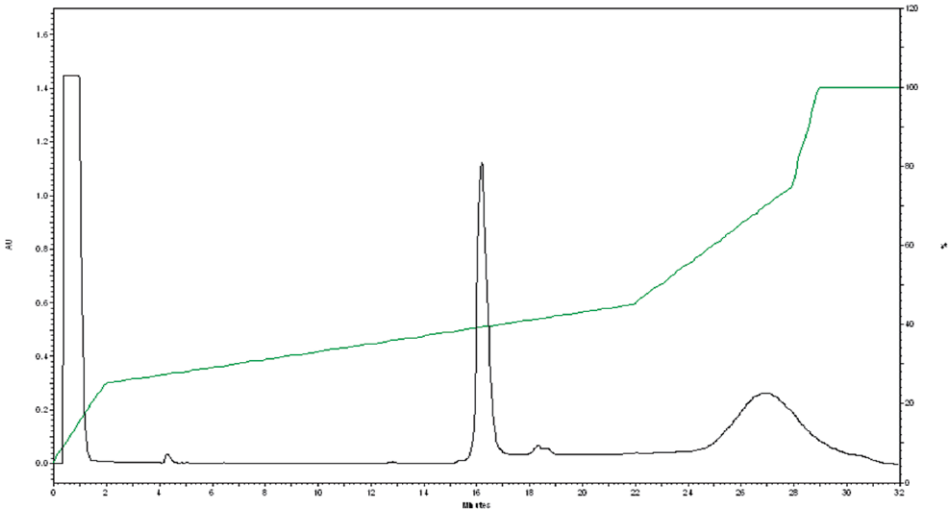


Fig.2 Digestion using a non-tryptic immobilized enzyme column.

Additional Workflows

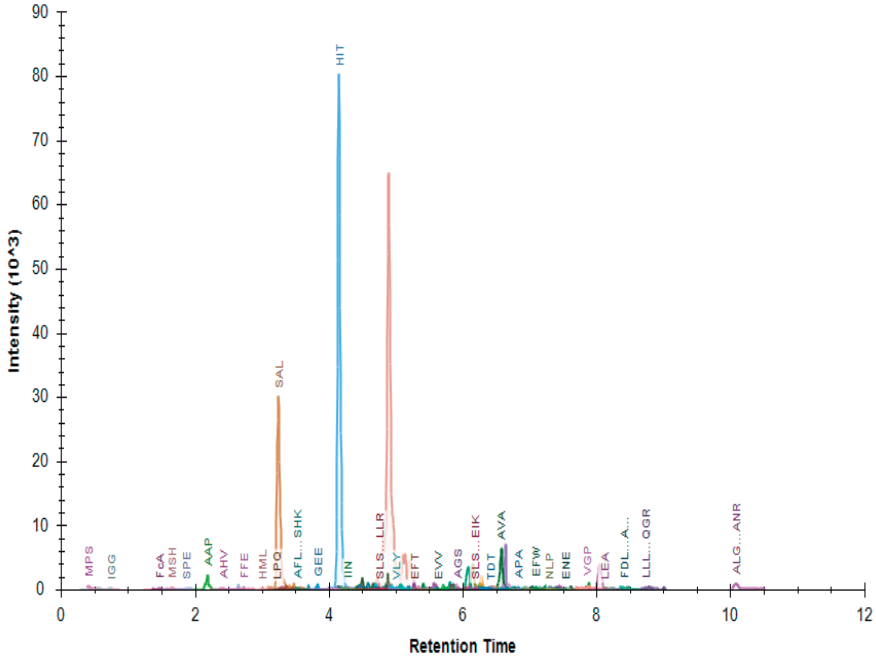
Immunochromatography

The use of affinity without digestion can be used to detect and collect intact proteins for further analysis. This can be used to isolate a single protein (right) or for mixtures and isoform analysis. This configuration simply requires the removal of the trypsin column and replacement with a union.



Desalt and RPC only (for MRM development)

When developing MRMs, rapid optimization is often required. To schedule MRMs, a predigested sample (see rapid digestion section) can be assayed using the workstation to optimize MRM parameters (retention time, collision energy, source parameters) without need for additional digestion time (e.g., right pane)



Digestion -LC-MALDI: See poster MP007 (at the ASMS 2015)

Future Directions

Depletion

By placing the trypsin column inline with the affinity column and using NoRA digestion buffer, abundant protein removal followed directly by digestion can be achieved.

Alternative chemistries

Using alternative chemistries, such as HILIC, coupled with a fraction collector, allows the multi-dimensional separation required for complex proteomics discovery LC-MS/MS workflows.

Summary

- The Perfinity Workstation was modified from its standard configuration to allow workflow-specific methodologies.
- Rapid digestion and desalting was coupled with MALDI for simple digest identification and characterization.
- Non-tryptic workflows were accommodated using modified digestion and wash buffers.
- Depletion and alternative chemistries can be applied as viable workflows.

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