# Automation of Sample Preparation and Buffer Exchange for Multi-Attribute Method

P. Nikki Sitasuwan[1]; Thomas W. Powers[2]; Tiffany Medwid[2]; Yuko Ogata[3]; Nancy S. Nightlinger[3]; Richard S Rogers[4]; Casey Snodgrass[5]; Pamela M. Quizon[1]; L. Andrew Lee[1]

[1]Integrated Micro-chromatography Systems, Inc, Irmo, SC; [2]Pfizer Inc., Chesterfield, MO; [3]Just - Evotec Biologics, Seattle, WA; [4]Bristol-Myers Squibb, Seattle, Washington; [5]Hamilton Company, Reno, NV

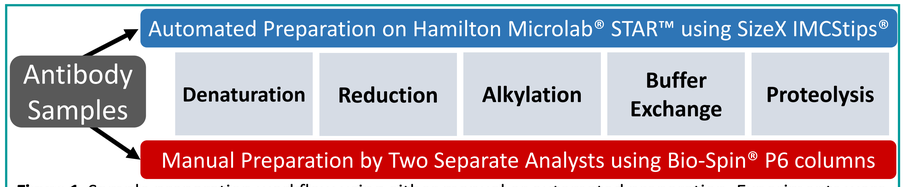



**Figure 1.** Sample preparation workflow using either manual or automated preparation. Experiments were performed at two independent sites using antibody stock with concentrations ranging from 1 – 10 mg/mL.

## INTRODUCTION

- Multi-Attribute Method (MAM) – extension of peptide mapping applied to the characterization of a biotherapeutic; leverages advances in ultrahigh-performance liquid chromatography coupled to mass spectrometry (UHPLC-MS) and data processing software.
- The combination of these technologies via MAM allows the simultaneous detection, identification, quantitation, and monitoring of molecular attributes of biotherapeutics.
- Current sample preparation for MAM relies on manual buffer exchange to remove excess denaturant and accelerate subsequent trypsin digestion efficiency.

## GOALS

- To compare manual sample preparation using Bio-Spin P6 spin columns vs. automated sample preparation with SizeX IMCStips on a Hamilton Microlab STAR
- To evaluate robustness and reproducibility of the automated method by testing at two independent sites
- To collect precision data from three separate timepoints to determine consistency of the automated sample preparation method

## MATERIALS AND METHODS

- SizeX IMCStips® were provided by IMCS. For manual buffer exchange, Bio-Spin® P6 columns (Bio-Rad) were used.
- Antibody stocks (1 to 10 mg/mL) were denatured, reduced, and alkylated
- The denatured protein samples (0.25 to 1 mg/mL) buffer exchanged using Bio-Spin P6 columns (manual) or SizeX IMCStips (automated)
- Desalted antibody was digested with trypsin to generate peptides for MAM analysis. Peptide samples analyzed on Thermo Q Exactive plus; data processed in BioPharma Finder. Known critical quality attributes were quantified and new peptide peaks were screened.

## RESULTS

### SITE ONE

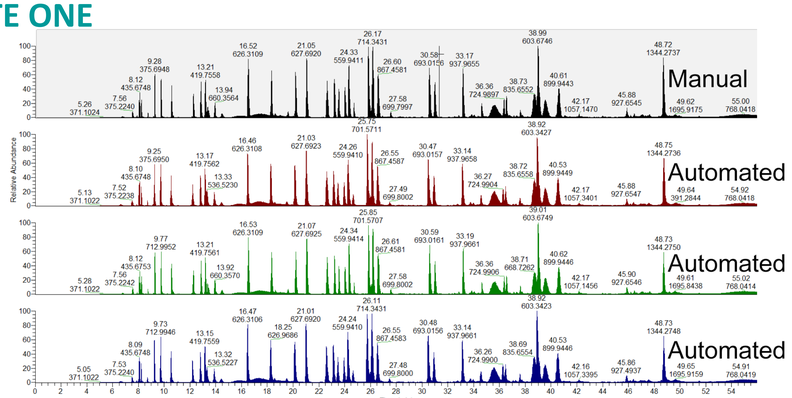



**Figure 2.** Total ion chromatograms (TICs) of peptide samples from manual and automated methods using 1 mg/mL antibody stock.

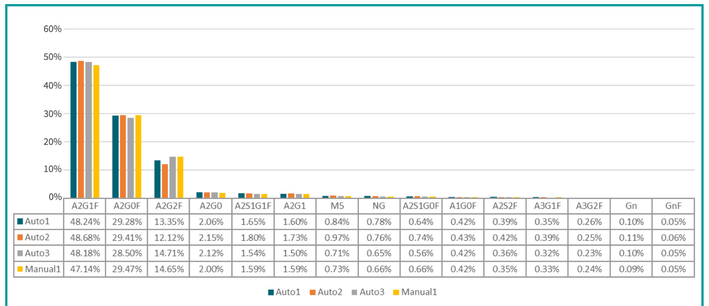



**Figure 3.** Fc glycosylation patterns of automated samples compared to manual samples.

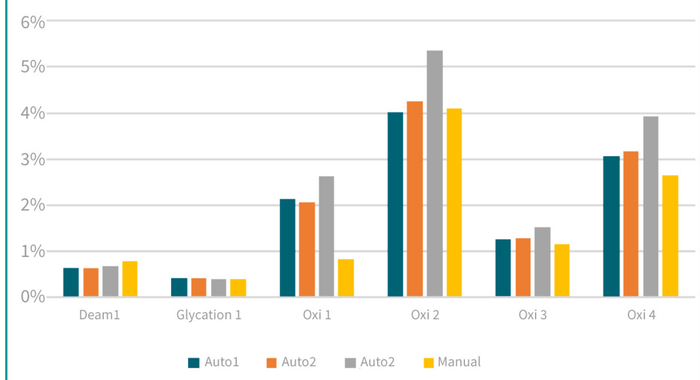



**Figure 4.** Modifications observed in automated samples compared to manual samples (Deam: deamidation, Oxi: oxidation).

### SITE TWO

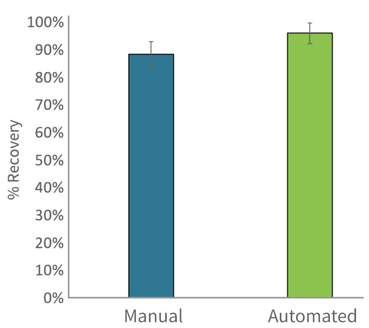



**Figure 5.** Antibody recovery from manual and automated MAM using Bio-Spin P6 columns and SizeX IMCStips, respectively (n = 4).

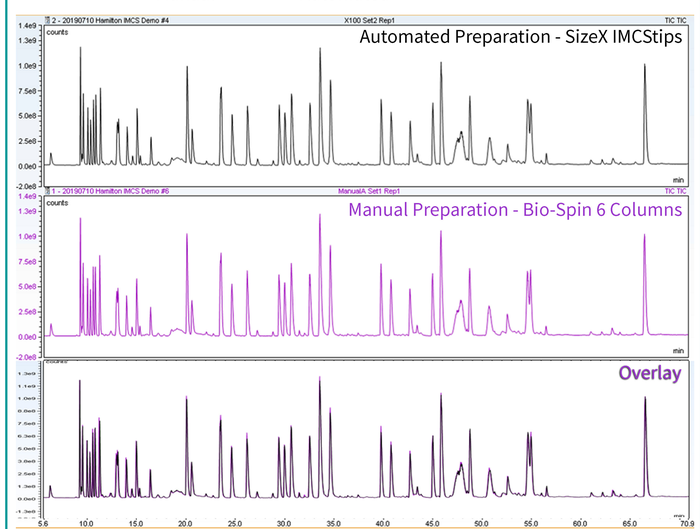



**Figure 6.** Total ion chromatograms (TICs) comparing automated (SizeX IMCStips) and manual preparation (Bio-Spin P6 Columns).

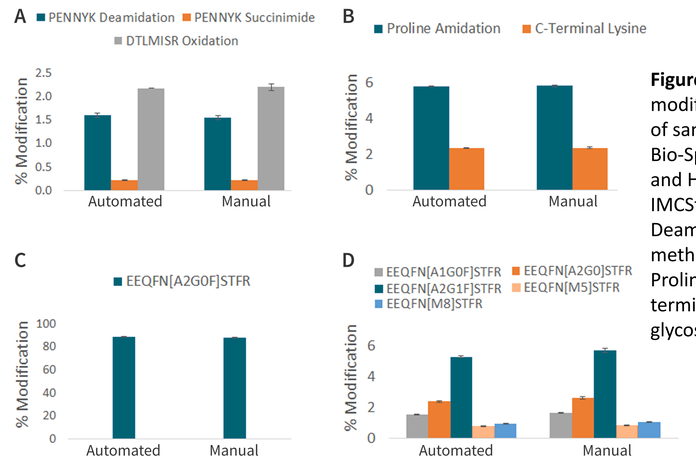
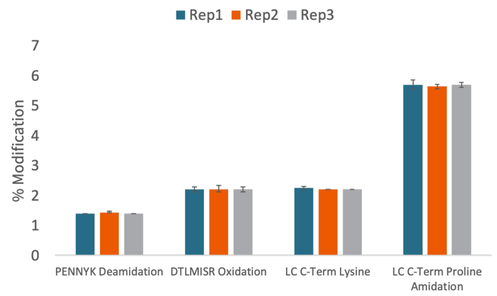



**Figure 7.** Comparison of modification percentages of samples prepared using Bio-Spin P6 columns (manual), and Hamilton STAR with SizeX IMCStips (automated). **(A)** Deamidation, succinimide and methionine oxidation. **(B)** Proline amidation and C-terminal lysine. **(C)** Fc glycosylation patterns (n = 4).

***Automated Prep***
- *3 replicates prepared at 3 separate timepoints*
- *4 samples per replicate*




***Manual Prep***
- *4 preparations*
- *4 analysts*
- *5 test dates*
- *10 injections*

***Automated Prep***
- *5 preparations*
- *4 test dates*
- *19 injections*

**Figure 8.** Precision of automated MAM sample preparation performed at three different timepoints with 4 replicates at each timepoint.

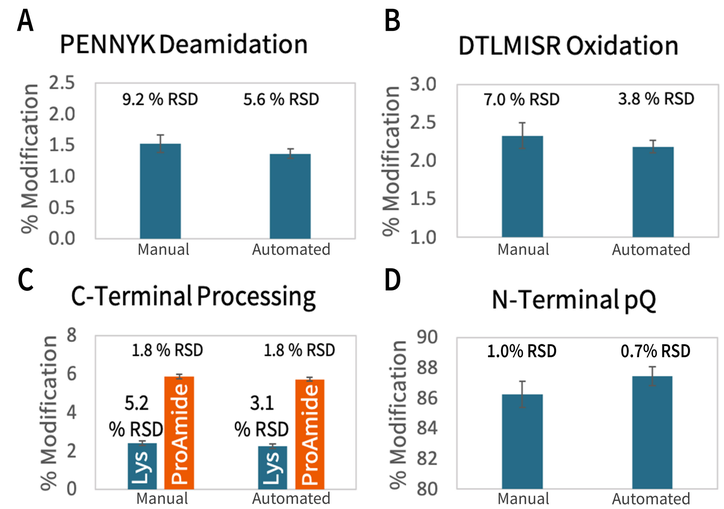



**Figure 9.** Reproducibility of automated MAM sample preparation compared to manual preparation.

## DISCUSSION & CONCLUSION

Automated MAM sample preparation utilizing SizeX IMCStips on Hamilton STAR liquid handling showed comparable precision and improved reproducibility over manual preparation. Automating tedious and repetitive sample preparation is a promising improvement for obtaining accurate and reproducible data for monitoring critical quality attributes of biotherapeutics. Furthermore, it could also serve as a platform to systematically optimize preparation conditions.

## ACKNOWLEDGEMENTS

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