Modular Automated Sample Extractions using IMCStips™ on Hamilton® Microlab STAR™ for High Throughput Protein Purifications

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INTRODUCTION

Protein therapeutics continue to expand as a major class of new drug products. Research and development into proteins require a purification processes using a range of resins. Integrated workflows with IMCStips demonstrate purification of different proteins using different INtip™ chemistries and the flexibility of the Hamilton Microlab STAR to achieve high throughput workflows with minimal human intervention.

- Automated protein A (MabSelect SuRe) INtip purification and buffer exchange in 2 hours.
- Peptide mapping using denaturation, reduction, alkylation and trypsin digestion.
- Examples of modular programs to run for small proof of concept experiments 8-24 samples to larger scale sample processing 96 sample batches.

METHODS

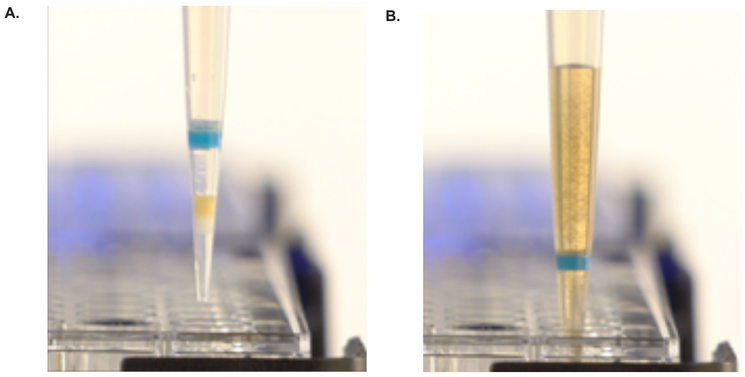


*Figure 1. Dispersive pipette extraction is a novel solid phase extraction process that leverages turbulent mixing inside a tip (**Figure 1A**). The loose resin contained within the tip is dispersed as the liquid sample is aspirated (**Figure 1B**). The extraction process occurs as the liquid sample is pipetted allowing faster and more efficient extraction.*

Modular programs on Hamilton Microlab STAR:

- Affinity purification using Ni-IMAC for polyhistidine tagged proteins.
- Antibody or Fab fragment purification using Protein A, G, or L.
- Biotinylated target enrichment using streptavidin resin.
- Size exclusion chromatography using SizeX.

Example of pre-templated deck layout for implementing INtip chemistries *(Figure 2A)*. Graphic user interface with variable commands to alter mixing cycles and volumes at each extraction step *(Figure 2B)*.

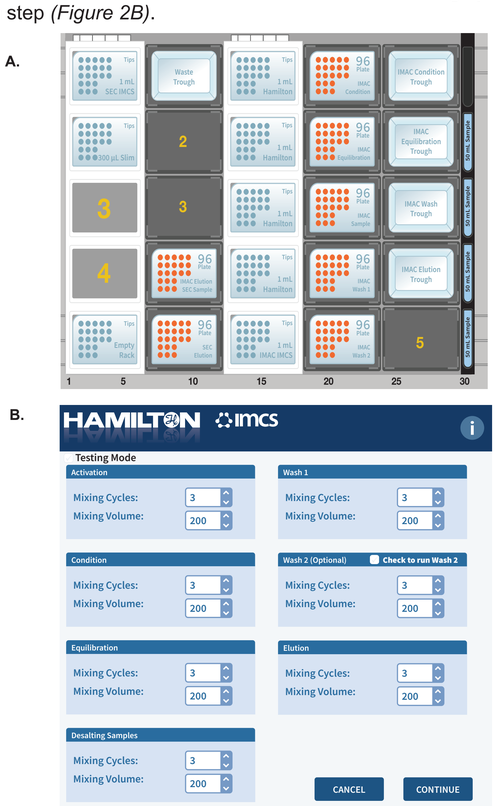
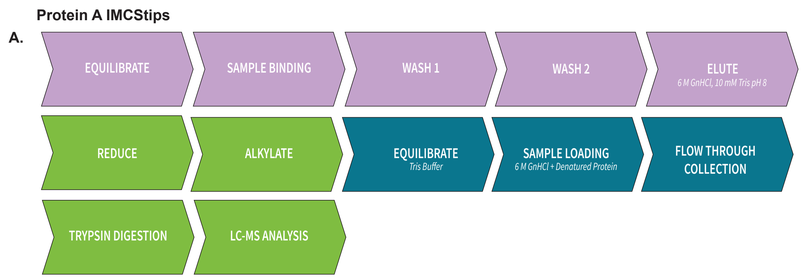
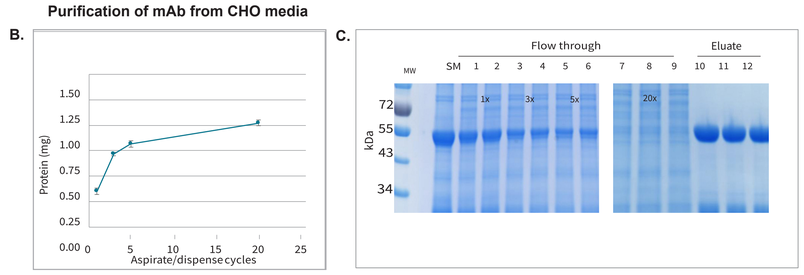


***Figure 2.** Example of pre-templated deck layout for implementing INtip chemistries (Figure 2A). Graphic user interface with variable commands to alter mixing cycles and volumes at each extraction step (Figure 2B).*

RESULTS

At Line Multi-Attribute Monitoring





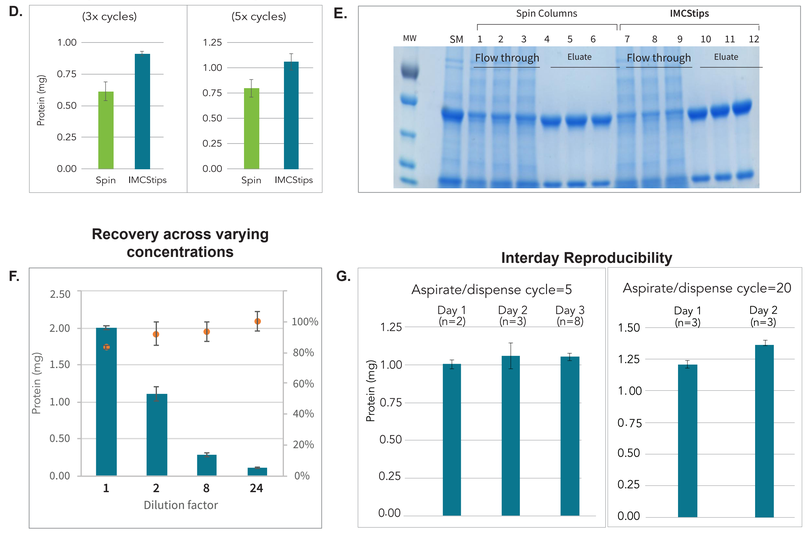


***Figure 3**. The workflow of at-line multi-attribute monitoring on Hamilton STAR platform using a combination of Protein A IMCStips and SizeX **(A)**. Purified antibody is denatured, reduced, alkylated, then desalted using SizeX, a size exclusion chromatography tip. Sample is trypsinized for final LC-MS injection. Purification of a single lot of CHO cell fermentation using MabSelect SuRe LX resin by IMCStips™. Increasing aspirate/dispense cycles increase the yield as indicated by protein quantification by UV-Vis **(B)**, and indicated by the depletion of the heavy and light chains in the flow through media **(C)**. The eluates are from 20x aspirate/dispense cycles using elution volume of 400 µL 0.1 M NaOAc pH 3.3. Each cycle is a duplicate run except for 20x flow through and the eluants were done in triplicates. For comparison, the spin method utilized 100 x g RCF for 5 minutes and flow through samples were re-applied to the column either 3x or 5x. Spin format consistently yielded less mAb than tip-based format, as indicated by both UV-Vis **(D)** and by SDS-PAGE **(E)**. Serial dilutions of CHO media was prepared using 0.1% BSA in 1x PBS, pH 7.4 to assess capacities and recoveries across the different concentrations **(F)**. The enrichment of mAb from different cell media were tested over 3 days using different number of tips and two difference aspirate/dispense cycles **(G)**.*

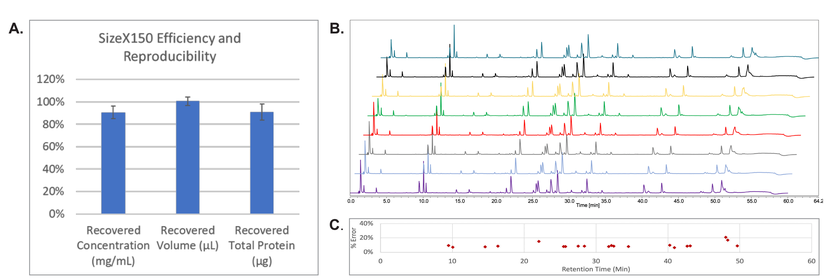
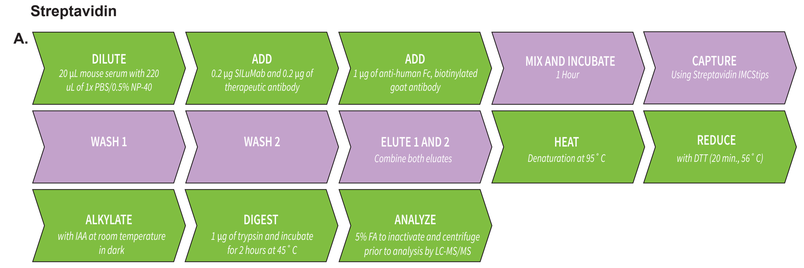
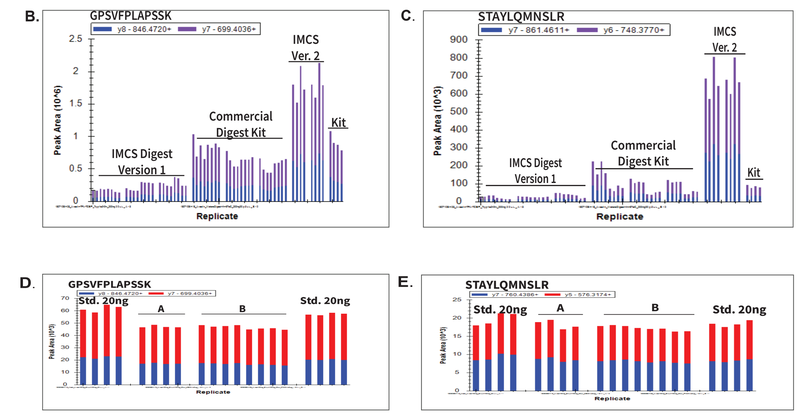


***Figure 4**. Monoclonal antibody at a final concentration of 1 mg/mL was denatured using 6 M guanidium hydrochloride. Next, it was reduced with dithiothreitol and alkylated with iodoacetic acid, respectively. 100 µL of the antibody was loaded onto IMCStips SizeX100 to remove denaturing agents for subsequent tryptic digestion. **(A)** Average percentages of recovered antibody concentration, volume, and total antibody amount (n=20). Error bar shows ± 1 S.D. **(B)** After the digestion, the sample was analyzed on HPLC-UV. Eight representative A280 chromatograms are overlaid. **(C)** Percent error of area integrations of 8 replicates.*

Therapeutic Drug Monitoring





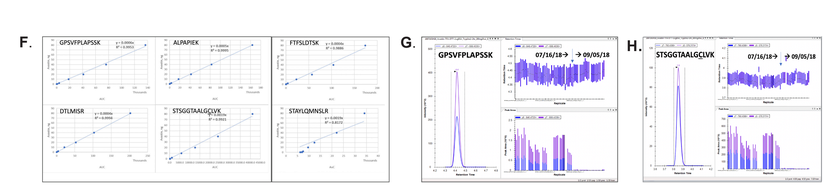


***Figure 5.** The workflow scheme for automating antibody quantification in pharmacokinetics study. **(A).** The target therapeutic antibody is a humanized IgG and the sample matrix is mouse serum. The therapeutic antibody was enriched by using anti-human Fc from goat with a biotin tag. The captured antibody was further enriched using streptavidin IMCStips on Hamilton STAR platform, followed by elution, denaturation, reduction/alkylation and digestion. **(B, C)** The modifications of the workflow and digestion times further increased the sensitivity by nearly 10-fold for the generic antibody peptides. **(D, E)** The recovery and consistency of the workflows were monitored with repeat injections using multiple IMCStips.Recovery was normalized against neat standards supplemented with trypsin digested BSA. **(F)** The four generic peptides and four unique signature peptides show linearity across a range from 10 pg to 80 ng. **(G)** The retention times and peak areas for the two generic peptides were continuously monitored to assess the robustness of the automated workflow.*

CONCLUSION

Affinity enrichment of various target analytes is a routine process that is implemented throughout the early to late research phases. The work shown here is based on automation and high throughput purification, but still providing room for flexibility to adjust the process for varied number of samples, varying affinities and coupling multiple workflow processes such as buffer exchange after affinity purification. The high consistency and flexibility are key points when implementing these workflows for purification of tagged proteins with Ni-IMAC, immunoglobulins from cell media using Protein A immobilized resin, pulling down biotinylated targets with streptavidin beads or automating the buffer exchange process with size exclusion chromatography. Ultimately, IMCStips coupled to the Hamilton Microlab STAR automated liquid handling system has multiple modalities that can provide flexibility, consistency and evolutionary compatibility as the needs of the scientist change over time.

REFERENCES

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