Automated Desalting and Buffer Exchange of Human IgG Protein in 6M Guanidine

HIGHLIGHTS: Process 96 samples in < 15 minutes

SEC

PURPOSE AND OBJECTIVES

- Demonstrate the utility of INTip Size Exclusion Chromatography (SEC) for the purification of Human IgG protein from 6M guanidine.
- Recover greater than 80% of IgG with undetectable guanidine in final elution with a 1:1 ratio of sample to elution volume.
- Show consistent column swelling resulting in low % RSD values.
- Demonstrate the benefits of patent-pending INTip Swelling and how the technology simplifies SEC workflows.

INTRODUCTION

DPX Size Exclusion Chromatography (SEC) Tip Technology incorporates traditional size exclusion chemistry in a pipette tip for an automated and efficient group separation method for removal of salts, buffer exchange, and protein purifications. High salt content is necessary in many workflows for the denaturation of proteins for downstream analysis. Unfortunately, salt can cause detrimental effects if present during final analysis steps. Size exclusion chromatography is utilized in the purification of proteins from their high salt content environments and exchanging them into a required buffer for analysis. DPX SEC tips are optimized to provide efficient recovery of target proteins above the media cutoff (1500 Da or 5000 Da).

Immunoglobulins are glycoproteins called antibodies which are produced when plasma cells work to mediate immunity by binding to specific antigens. This characteristic makes monoclonal antibodies (mAb) a commonly used drug delivery system for clinical therapeutics. Among the five isotypes of immunoglobulin antibodies found in human serum, Immunoglobulin G (IgG) is most common, representing 75% of all immunoglobulins in humans. IgG is also a primary focus for drug discovery and development due to it's long half-life. Around 160,000 Da in molecular weight, IgG antibody has been chosen to validate the use of SEC Tips for these common applications in the pharmaceutical industry.

METHOD

This automated method was performed with a Hamilton STAR automated liquid handling system (ALH). SEC Tips were first pierced on the deck with a piercing tool shown in Figure 1. The piercing step adds less than 10 minutes to the method. Automated piercing and the DPX patent-pending process for INTip Swelling eliminates all pre-ALH steps such as slurry preparation, column packing, centrifugation, and manual cap or plug removal.

Figure 1. Piercing tool from DPX Technologies designed for compatibility with Hamilton Robotics platform for on-deck piercing which replaces manual cap or plug removal.

For automated INTip swelling, SEC Tips over-aspirate swelling buffer and dispense residual buffer to create a perfectly equilibrated bed. The sample containing 1 mg/mL IgG human protein antibody and 6M guanidine is then added to the bed. The ALH then picks up the DPX SEC tip to dispense the sample into the SEC bed, displacing the swelling buffer and delivering it to a waste well plate. Once the 100 µL sample has been dispersed across the SEC bed, 165 µL of PBS is added to the top of the bed. The ALH then picks the SEC tips back up and dispenses 65 µL of void volume to the waste well plate. The protein is then eluted by dispensing the remaining 100 µL of PBS into an elution plate. The piercing, automated INTip Swelling and method was performed in under 15 minutes. Figure 2 shows the method schematic.

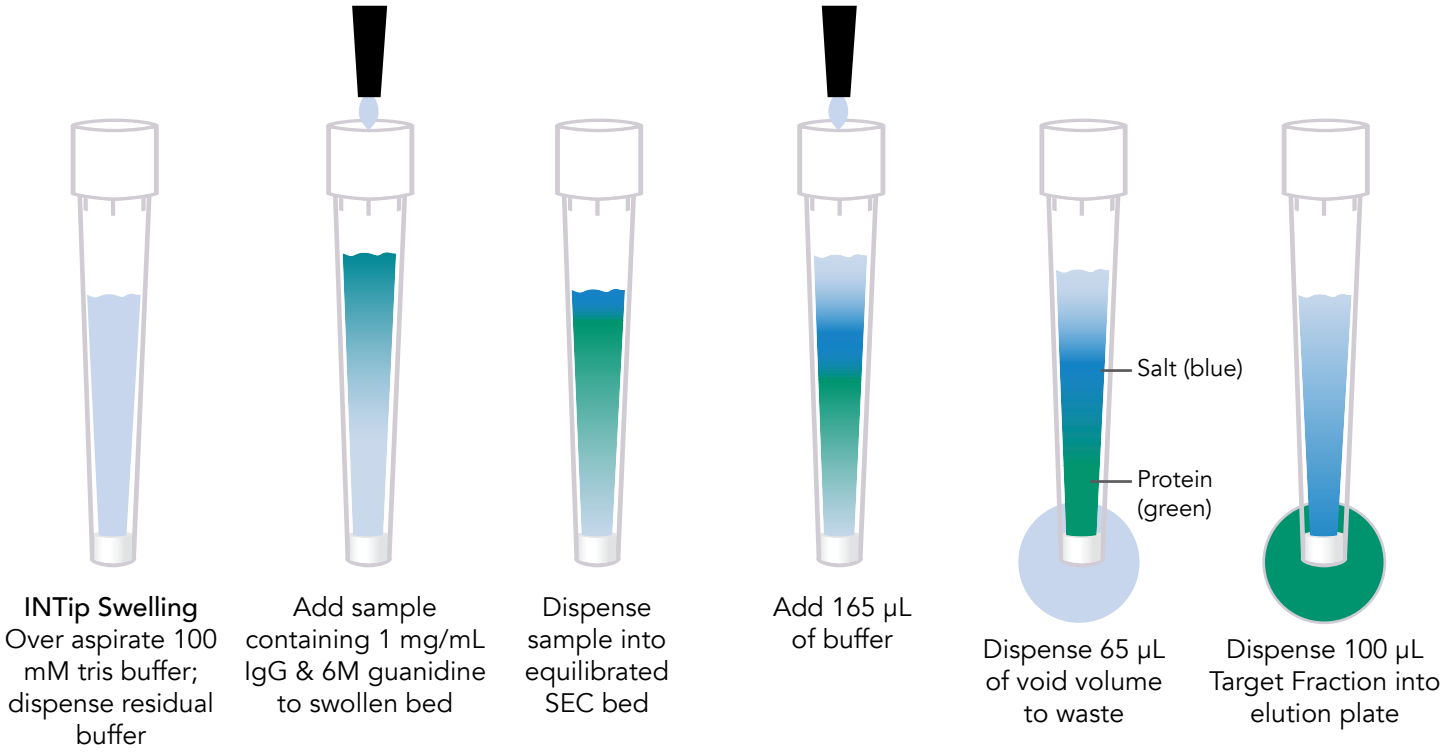

Figure 2. Schematic represents workflow for INTip Swelling and size exclusion method for purification of Human IgG.

RESULTS AND DISCUSSION

This method provides greater than 85% recovery of IgG human protein antibody while removing the salt (as shown in Figure 3), demonstrating that this technology and method can be integrated into a large variety of protein isolation applications. With just a 100 µL sample volume and 100 µL elution volume, DPX SEC tips minimize dilution of analytes of interest. Recovery can be further maximized while maintaining the undetected salt content in final solution by eluting with 50 µL more. Although 6M guanidine was used in this workflow, DPX SEC tips can be utilized for common buffer exchanges up to a concentration of 8M guanidine.

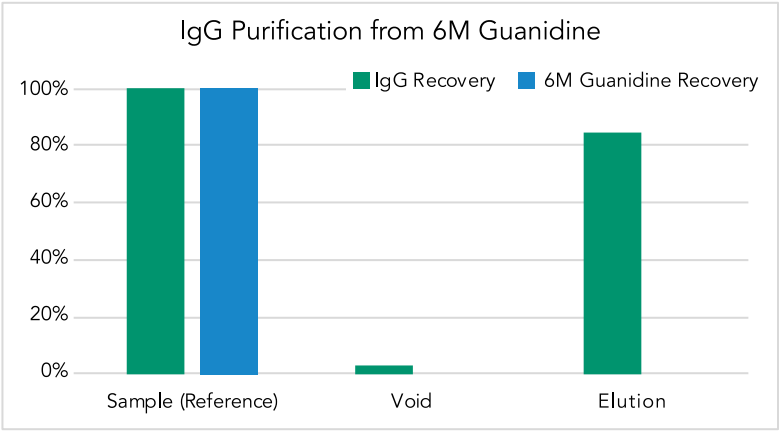

Figure 3. SEC Tips provide greater than 85% recovery of IgG human protein in 6M guanidine, a high-salt content environment. Guanidine is not detected in target elution, and is still retained on the SEC bed, giving efficient buffer exchange and purified protein for downstream analysis.