# Automated mAb Workflows: Combining Multidimensional (Multi-D) Purifications with Product Analysis

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## Abstract

The purification of biologics, and specifically monoclonal antibodies, frequently involves multiple chromatography columns and buffer systems. The prevalence of small-batch biologic production allows the use of smaller chromatographic columns, which can be more amenable to completely automated purification. A single Multi-D method combining both purification and analysis on the NGC™ Chromatography System allows for completely hands-free, reproducible, and high-fidelity purifications without scientist intervention. By automating purification, in-line analysis can be added at any point in the workflow to investigate a small portion of the sample before saving the rest or continuing the purification.

Here, a typical mAb protein purification and analysis workflow is fully automated followed by host cell protein (HCP) quantitation with ELISA. A single method combines a Protein A and desalting column (for neutralization) in tandem with an ion exchange pair in tandem before aggregation analysis using size exclusion chromatography (SEC). Final HCP-ELISA quantitation demonstrates the ability to generate a single purification method on the NGC Chromatography System that is as effective as doing each purification step individually.

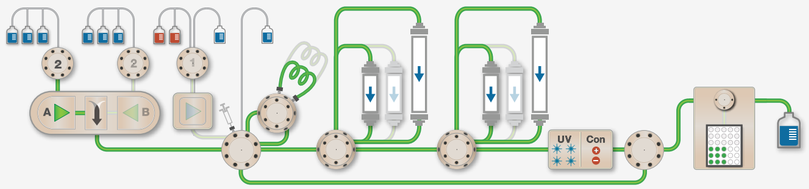
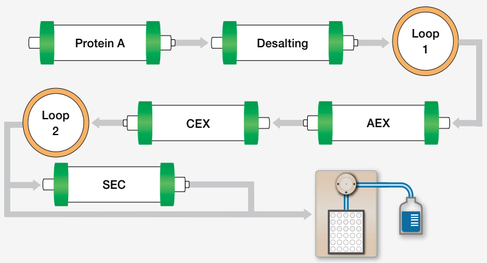



**Fig. 1. Schematic of the Multi-D NGC System configuration.** This schematic represents an NGC Discover™ 10 Pro System with two additional column switching valves. Column switching valve 3 is plumbed as a loop valve replacing the static loop on the inject valve. A fluidic line from outlet valve port 2 is plumbed into the injection port of the inject valve. The columns used in the method can be set up for individual runs or as tandems on column switching valves 1 and 2. All of the static loops used in the Multi-D method are plumbed on the loop valve.

### mAb Purification Workflow

**A. Traditional Sequential Workflow**

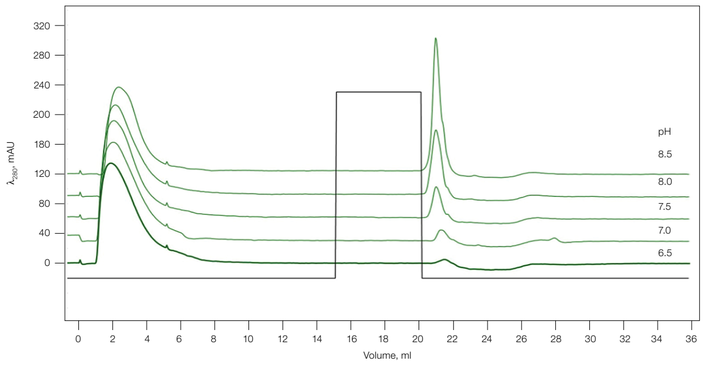

**B. Automated Multi-D Workflow**




## IEX Tandem Optimization

During method development, 1 mg of mAb in Chinese hamster ovary (CHO) tissue culture fluid (TCF) was loaded onto the columns. The Protein A column binding and elution conditions were previously optimized (Bio-Rad Laboratories, bulletin 6694). Most mAbs have an acidic isoelectric point and thus do not bind to an anion exchange column (AEX) at near neutral or lower pH because they carry an overall positive charge. The AEX column thus served to bind negatively charged HCPs and host cell DNA. The unbound mAb in the AEX flowthough was then loaded onto a CEX column. To eliminate the need for buffer exchange between the two IEX columns, we needed to determine a pH that would allow the mAb to flow through the AEX column but bind to the CEX column (Figure 2). Subsequently, CEX conditions were optimized by %B scouting to generate a protocol that yielded a compact and concentrated mAb peak (Figure 3).

**A**




**B**

| pH | % Recovery |
|---|---|
| 6.5 | 98 |
| 7.0 | 96 |
| 7.5 | 91 |
| 8.0 | 81 |
| 8.5 | 68 |

**Fig. 2. AEX pH scouting. A**, pH scouting chromatogram overlay showing the use of the anion exchange column in flow-through mode at different buffer pH values. $A_{280}$ (—), %B (—). As the pH increases, in addition to increased HCP binding, more of the mAb binds to the resin. The anti-CHO HCP-ELISA data showed an order of magnitude decrease for every pH (data not shown), so protein recovery was used to drive the pH buffer selection of pH 6.5 (**B**).

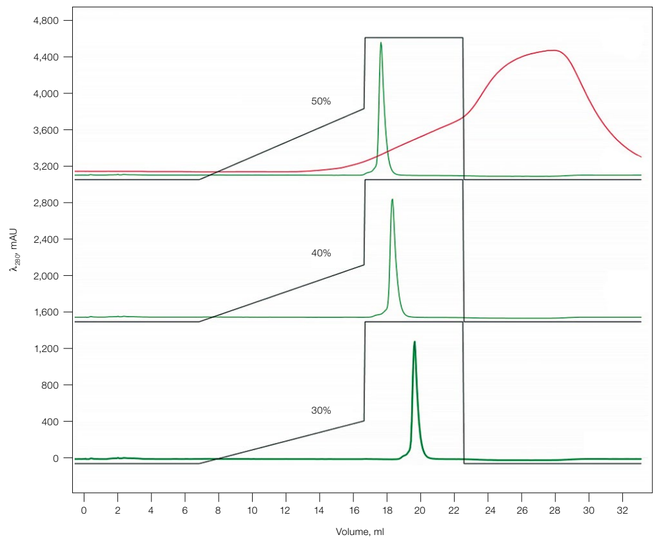



**Fig.3. CEX %B scouting.** All three separations showed undetectable levels of HCPs. 50%B elution yielded the most compact peak and was used for the Multi-D method. $A_{280}$ (—); conductivity (—); %B (—).

## SEC Analysis

Protein eluted from the CEX column using the automated Multi-D workflow was captured in a static loop. A small portion underwent analytical SEC aggregation analysis (Figure 4) while the majority was sent to the fraction collector for subsequent ELISA analysis.

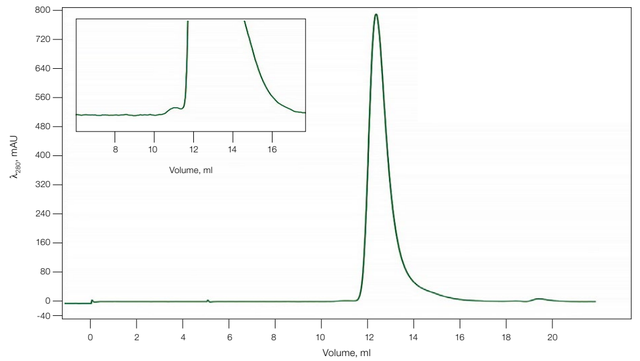
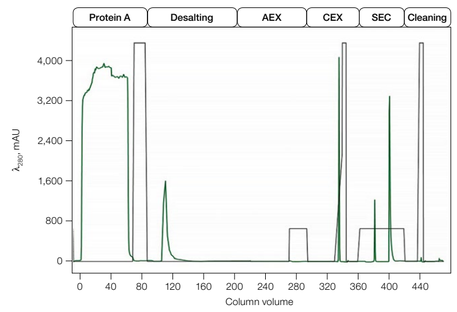



**Fig.4. SEC aggregation analysis.** A small portion of the pooled cation exchange fractions was injected onto the ENrich™ SEC 650 for aggregation analysis. The inset shows a magnified portion of the chromatogram demonstrating the separation of the leading higher aggregate species from the monomer peak. $A_{280}$ (—).

## Final Automated Purification and Analysis

Following optimization of the individual column purification steps, the NGC System was plumbed into a Multi-D configuration (Figure 1), and the optimized buffer and elution conditions from each run were combined into a single method within ChromLab™ Software. Final purity of the samples for the traditional sequential and automated Multi-D methods are indistinguishable (Figure 5B).

**A**




**B**

| | HCP Concentration, ppm | Error, ± |
|---|---|---|
| **Traditional Sequential** | | |
| TCF | 1,100,000 | — |
| Protein A | 32.86 | 10.51 |
| Desalting | 11.90 | 3.81 |
| AEX | 2.38 | 0.76 |
| CEX | 0.71 | 0.23 |
| **Automated Multi-D** | | |
| TCF | 1,100,000 | — |
| CEX | 1.09 | 0.38 |

**Fig.5. Automated Multi-D purification and analysis. A**, Multi-D chromatograms showing $A_{280}$ (—) and %B (—). This automated single method runs two sets of tandem chromatography purifications resulting in undetectable HCP contamination. In addition to the purification, aggregation analysis using analytical SEC was also carried out (the first SEC peak) before sending the rest of the purified sample straight to the fraction collector (the second SEC peak); **B**, the final purity of samples from traditional and automated Multi-D purifications was indistinguishable.

## Conclusion

The NGC Chromatography System and ChromLab Software allowed the development of a robust, high-quality, and automated multicolumn chromatography purification utilizing Multi-D purification and SEC analysis. This automated Multi-D workflow yielded purified mAbs with HCP levels equivalent to those of mAbs purified using lower-throughput traditional sequential methods.

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