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Multi-column chromatography implementation and scale-up

Your Challenge

- You deal with increasingly challenging antibody purification throughput bottlenecks and need to increase the efficiency of your capture process.
- You need a straightforward proof of concept showing implementation and scale up of a multi-column chromatography process.

Our Solution

Octave and SkillPak Multi-Column Chromatography Platform

- Scalable systems, software, and columns

What was done?

- An existing purification process was transferred to a multi-column chromatography format at a 100 g pilot scale and scaled up to a 1 kg clinical scale.

What was the result?

- Resin volume and buffer savings were achieved by transferring to MCC, while repeatable impurity profiles were achieved with the scaled-up process.

Existing antibody purification processes can be transferred to a multi-column format and scaled up with the Octave BIO process development system and the Octave PRO GMP ready system. Catalent now considers MCC as an option of their process intensification offering alongside their GPEx® Lightning cell line development technology.

Your Benefit

Observe the measurable benefits of intensified purification with multi-column chromatography at a clinical scale.

Intensified purification of antibody therapies

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https://www.separations.eu.tosohbioscience.com/products/chromatography-instruments/multi-column-chromatography/octave-pro-system

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Application Note

Multi-Column Chromatography Implementation and Scale-Up to Clinical Scale with the Octave™ MCC Systems

Multi-column chromatography (MCC) is a purification technology with well-characterized benefits for bind-and-elute capture processes. Operating with multiple columns increases process efficiency and productivity, resulting in a reduction in column sizes, resin and buffer requirements, and overall lower process costs. Here, we present a case study demonstrating the development of an MCC process for the Protein A affinity step of a monoclonal antibody (mAb) purification on the Octave BIO system and the straightforward scale-up to a clinical scale using the Octave PRO system. This was a joint study conducted between Catalent and Tosoh, where a direct comparison was made of a typical batch process with that of an MCC process while maintaining the same buffer conditions. This project was conceived jointly between Tosoh Bioscience and Catalent and the study was performed at the Catalent.

Featured Technologies

Octave BIO Multi-Column Chromatography System

The Octave BIO is a bench-top MCC system based on the rugged, reliable, and patented Octave technology developed specifically for bio-separations. The system can run protein purification methods comprising up to 8 columns using a proprietary valve block, six pumps, and column valve block inlets and outlets. The flow path can accommodate up to a 300 mL/min flow rate. The system has integrated detectors for data recording, including four of each for UV, conductivity, and pH monitoring. Additionally, the system can be operated in a single-column mode with a sample injector for initial method development and resin scouting experiments. The functionality to execute scouting methods consisting of gradient elution is also available.

Octave PRO Multi-Column Chromatography System

The Octave PRO system is a CGMP-compliant MCC system for the purification of biologics at both clinical and commercial scale. Octave PRO has been designed as a direct scale-up system after process development on the Octave BIO, offering the same method-execution capabilities. With the ability to support up to 8 columns, a 2.5 L/minute flow rate and featuring a single-use flow path for quick process changeover and improved safety, the Octave PRO is designed for process versatility and agility for addressing the most challenging downstream bottlenecks. In addition, four single-use dual-channel UV, pH, and conductivity sensors enable complete process monitoring, and the 21 CFR part 11 compliant software allows operators process control in a CGMP environment.

Octave System Software

Accompanying every Octave MCC system are the Octave PROComposer™ method authoring software and MethodWizard™ process development tool. A unified method development and authoring platform, the MethodWizard allows for seamless method translation from batch to MCC, while the PROComposer can generate chromatography methods from development to process scales for all Octave systems.

Resins and Prepacked Columns

SkillPak™ PRO columns are the first clinical and commercial scale prepacked columns to feature shorter bed heights for faster flow rate (and shorter residence time) requirements of MCC. They are packed with TOYOPEARL® AF-rProtein A HC-650F resin to form a holistic solution for mAb purification when operated on the Octave MCC systems.

mAb Capture Process Transfer and Comparison to Batch

Experimental Conditions

Initial experiments were performed with self-packed TOYOPEARL AF-rProtein A HC-650F columns to transfer an existing batch mAb Protein A process to an MCC operation on the Octave BIO. Both batch and MCC processes were designed to purify a mAb produced from a CHO cell line with a titer of 6 g/L at different residence times (RT). According to a reference Catalent batch process, a single column (14 ×16 cm) was loaded and operated at a 4 minute residence time. However, due to the shorter bed heights of the MCC process columns (5.2 cm), the corresponding MCC process could operate at much faster linear velocities (0.5 min RT only).

MCC process design was facilitated by the Octave MethodWizard software and formalized into a method with the Octave PROComposer software. To minimize flowthrough losses from operating at such short residence time, we leveraged the benefits of the MCC technique by designing to run with a total of 8 columns, with 3 columns loading in series. The operating capacity for both processes was characterized, with the batch process loading capacity of 55 g/L determined by breakthrough experiments and the MCC process loading capacity of 65 g/L being calculated as a percentage (90%) of the resin's static binding capacity.

Each process was performed until approximately 100 g of mAb was loaded to compare batch versus MCC performance. This corresponded with one batch process executed over 2.7 hours, compared to 4 MCC cycles completed within 3.7 hours (*Table 1*). Product yield and quality were then evaluated separately through appropriate analytical tests.

Table 1. Comparison of key process parameters between batch and multi-column processes at the bench scale.

ProcessParameters	Batch	MCC
Columns	14 × 16 cm	3.5 × 5.2 cm
Number of Columns	1	8
Protein A resin volume	2.46 L	0.40 L
Buffer volume	97.5 L	58.4 L
Residence time	4 min	0.5 min
Total feed loaded	22.6 L, 136.0 g	17.6 L, 103.6 g*
Process time	2.7 h†	3.7 h

*Amount loaded during 4 cycles (steady state)
† Does not include pre-run sanitization

Results

Performance data comparisons between the batch and Octave BIO MCC processes are shown in *Table 2*. The MCC process is more efficient, offering a productivity of 63.5 g/L/h compared to a productivity of 19.2 g/L/h in batch mode. In terms of benefits derived from this increased efficiency, the resin volume needed to process approximately 100 g of mAb is reduced by 84% when transitioning from batch to MCC, demonstrating pilot scale throughput with bench scale resin and columns.

Table 2. Comparison of key performance and quality results between batch and multi-column processes at the bench scale.

Efficiency Results	Batch	MCC
Operating Capacity	55 g/L	65 g/L
Capacity Utilization	76%	90%
Yield	126.4 g, 93%	94.8 g, 91%
Productivity	19.2 g/L/h	63.5 g/L/h
Quality Results	**Batch**	**MCC**
Monomer Percentage	97%	97%

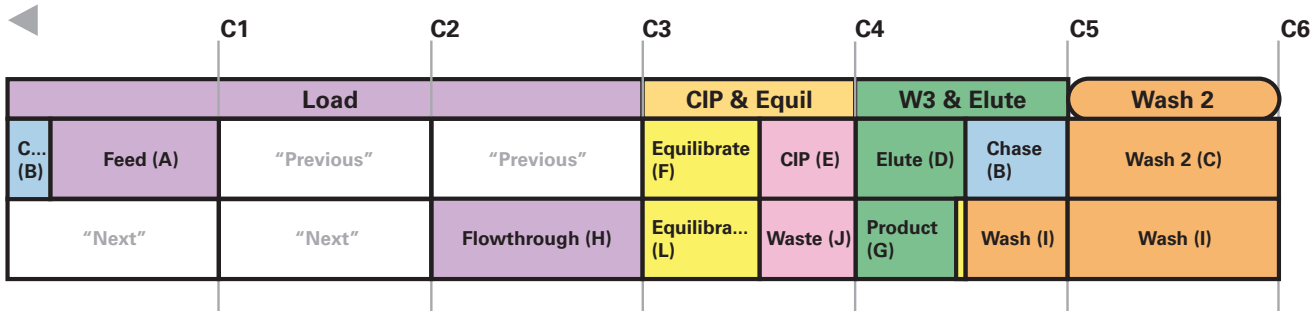
The MCC process also exhibits greater capacity utilization despite loading the resin at much lower residence times (0.5 minute). Operating with three columns in series in the load zone allows the resin to reach 90% saturation, while loading in a batch setting achieves only 76% saturation. As a result of this increased capacity utilization and reduced resin volume, the Octave process also exhibits buffer usage reduction of more than 40% while maintaining a similarly high yield. Overall, increased productivity, a smaller process footprint, and reduced buffer consumption was achieved using the MCC process (compared to the batch process). Transferring the process from batch to MCC does not affect product quality, as product monomer levels (characterized by SEC) remained consistent between the batch and MCC processes.

Clinical Scale with the Octave PRO

Experimental Conditions

After demonstrating the efficiency and productivity improvements of the MCC process at a 100 g scale, a 10x scale up to a kilogram scale process was jointly designed between Catalent and Tosoh and executed on the Octave PRO system at Catalent. The residence time (0.5 minute) and the operating capacity (65 mg/mL) of the MCC process were kept constant in the experiments described above.

Figure 1. Process flow diagram of the 6-column scale up MCC configuration.



In this configuration there are three columns in the capture (Feed) zone (columns 1–3), one column in the Wash 2 zone (columns 6), and one column each in the W3 & Elute and CIP & Equilibrate zones (columns 5 and 4, respectively). Each process step has a dedicated pump and inlet and a defined outlet. During regular time intervals programmed in PROComposer, specified inlet and outlet ports are switched to the next process step via a series of valves arrayed in a compact three-dimensional valve block (e.g. Octave PRO System) to produce a continuous cycle.

Utilizing the same MethodWizard and PROComposer software, a six-column method (*Figure 1*) for increased productivity, was designed utilizing the same TOYOPEARL AF-rProtein A HC-650F resin, obtained in a prepacked SkillPak PRO format (14 × 5.1 cm). Because of the similarity between the process design, monitoring, and control software, the small-scale (Octave BIO) and large-scale (Octave PRO), process scale-up was straightforward.

The process parameters of the scaled-up experiments are summarized in *Table 3*. The two runs presented here were executed with the designed process, each processing a 250L bioreactor at similar expression levels (5.6 and 6.2 g/L) for three steady state cycles. After Protein A capture, the eluate was neutralized to pH 6.8 - 7.2, and the product impurity profile (aggregation, HCP, DNA) and quality (monomer %) were assessed through offline analytical testing.

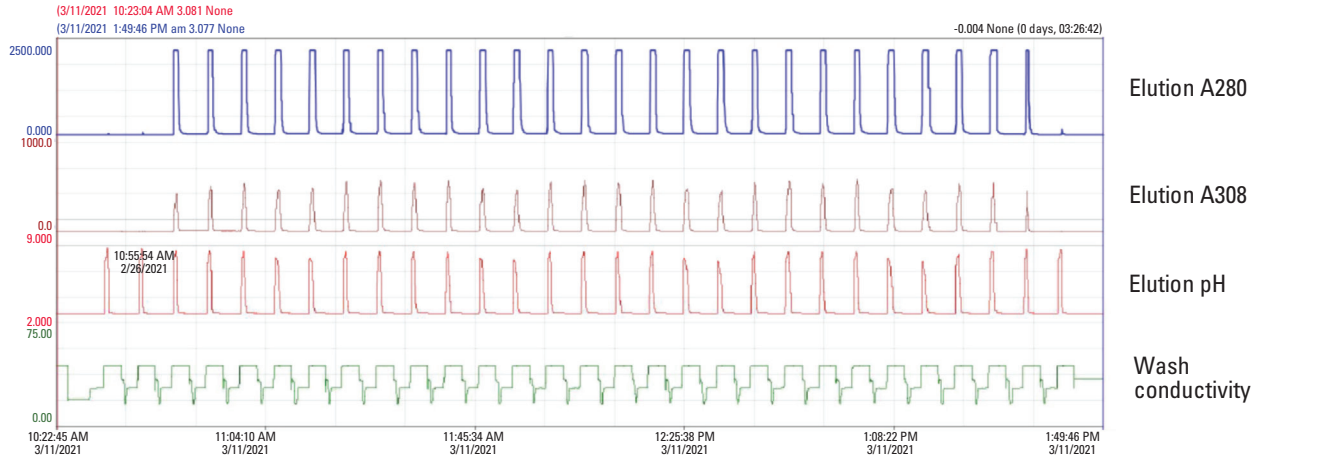
Table 3. Key process parameters for the scaled-up multi-column processes.

Process Parameters	Run 1	Run 2
Column Size	14 × 5.1 cm, 0.785 L	
Number of Columns	6	
Total resin volume	4.71 L	
Residence time	0.5 min	
Expression Levels	5.6 g/L	6.2 g/L
Total Feed Loaded	206.4 L, 1123.4 g	185.3 L, 1148.6 g
Process time	3.4 h	3.1 h

Figure 2. Operation of the Octave PRO at Catalent facilities.

Figure 3. The SkillPak PRO columns installed for the scaled-up MCC process.

Figure 4. Process monitoring sensor profiles as viewed on the Octave PRO.



Results

The Octave PRO, along with SkillPak PRO columns, shows consistent column performance during the execution of the kilogram scale MCC process. Qualitatively, the sensor profiles at steady state for Elution UV, Elution pH, and Wash conductivity are consistent over four cycles (*Figure 4*).

The measurable results of the two runs are summarized in *Table 4*. The offline analysis of product quality confirms the consistent performance of the MCC purification process. Aggregate impurities were at or below 0.5% for both runs, while host cell protein and DNA levels were reduced consistently by about 1.5 logs for both runs, showing replicable impurity clearance. Product monomer levels remained consistently around 95% as well.

Table 4. Key process parameters for the scaled-up multi-column processes.

Scale Up Results	Run 1	Run 2
Yield	1,041.5 g, 93%	1,055.5 g, 92%
Steady State Productivity	76 g/L/hr	83 g/L/hr
% aggregate	0.5%	<0.5%
% monomer	95%	94%
HCP log reduction	1.59	1.55
DNA log reduction	1.49	1.44

Regarding process productivity, both processes maintained the efficiency gains of transferring a batch process to MCC and even exceeded them vs. the scale-down model. The slight variation in titer between the two bioreactors resulted in slightly higher productivity in the second run than the first. Higher titer feedstocks can be purified with greater efficiency in an MCC process due to the reduction in load time to column saturation. The MCC process enables ~1 kg of product to be processed in under 3 hours.

The ability to use up to eight columns on the Octave PRO allows processing feedstocks with an even higher titer, reducing future downstream bottlenecks as titers continue to increase.

During the scale-up process, an important observation was made: the 1 kg MCC process maintained the same column diameter (14 cm ID) as the scaled-down 100 g batch process. The utilization of MCC allowed for the addition of more columns and increased process productivity, enabling the processing of significantly larger product masses, in this instance, a tenfold increase, while still maintaining smaller column dimensions. This advantage of MCC becomes particularly significant when considering the non-linear cost increase associated with larger prepacked columns compared to smaller column sizes.

Summary

The Octave BIO and PRO systems simplify the transition from a legacy batch purification process to an MCC process. Scaling up the process from a 100 g pilot scale to a 1 kg clinical scale on the Octave PRO was executed with the same PROComposer software tool that automatically scales the MCC process steps according to the mAb titer and column dimensions entered.

Compared to the reference batch process, the implementation of an Octave MCC system for this specific mAb capture process results in improvements in process productivity, resin utilization, and buffer consumption. These enhancements led to reductions of over 80% in resin volume and over 40% in buffer usage, respectively.

Overall, upwards of 1 kg mAb was produced in about 3 hours, all with only 4.71 L of resin. Catalent now considers MCC as an option of their process intensification offering alongside their GPEx® Lightning cell line development technology.