Continuous chromatography in downstream processing of a monoclonal antibody

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In this study, periodic counter current (PCC) chromatography and straight-through processing (STP) technologies were evaluated in a continuous three-step monoclonal antibody (MAb) purification process.

MAb capture was performed using MabSelect SuRe™ LX protein A chromatography medium (resin) in a three-column PCC (3C PCC) setup on the ÄKTA™ pcc 75 chromatography system. To assess robustness of the setup, 10 cycles were performed. The results showed consistent yield and purity over time. Using the 3C PCC setup, the capacity utilization could be increased by 56% as compared with an equivalent batch run.

The capture step was followed by two polishing steps in an STP setup on the ÄKTA pure chromatography system. Capto™ S ImpAct and Capto adhere media in serially connected columns with an intermediate in-line conditioning step were used. Two setups were evaluated, where the first Capto S ImpAct polishing step was run in either bind-elute (B/E) or flow-through (FT) mode. The second Capto adhere step was performed in FT mode in both setups. Results from both setups showed similar yield and purity as what can be expected from a traditional setup performed in batch runs.

Introduction

Continuous processing is well-established in many industries. At present, there is an increased interest in continuous processing also for biopharmaceutical manufacturing. Process intensification by implementing continuous or semi-continuous downstream processes in MAb production, for example, can contribute to significant cost-savings and improved throughput. Continuous processing also offers the possibility of increased automation of the process. As alternatives to traditional batch processes, new emerging technologies such as PCC and STP can be used.

Compared with a traditional batch setup, the capacity of the chromatography medium can be utilized to a greater extent in a multi-column PCC setup, with reduced chromatography medium volume requirements, reduced buffer consumption, shorter processing times, and potential cost savings as results (1). It has been shown that the cost savings achieved using PCC are more significant for early-stage clinical manufacturing than for later-stage clinical or commercial manufacturing (2).

Concept of PCC

In a PCC setup, columns are switched between the loading step and non-loading steps comprising column wash, elution, cleaning in place (CIP), and equilibration. At a predefined level of breakthrough, the primary column in the loading zone is disconnected from the loading zone and the load is redirected to the next column. In parallel, the disconnected, saturated column will be washed, eluted, and regenerated.

ÄKTA pcc 75 supports continuous chromatography in a 3C PCC setup. To fully utilize the potential of this setup, the non-loading steps should be shorter or equal to the time for loading of a column. One cycle equals three loadings, one on each column.

Loading can be controlled either statically (based on time) or dynamically (based on UV absorbance). Dynamic control enables adjustments with regard to load in the case of changes in feed composition and/or chromatography medium capacity. Dynamic control is described in detail in application note 29169455 (3). A schematic overview of a 3C PCC setup is given in Figure 1.

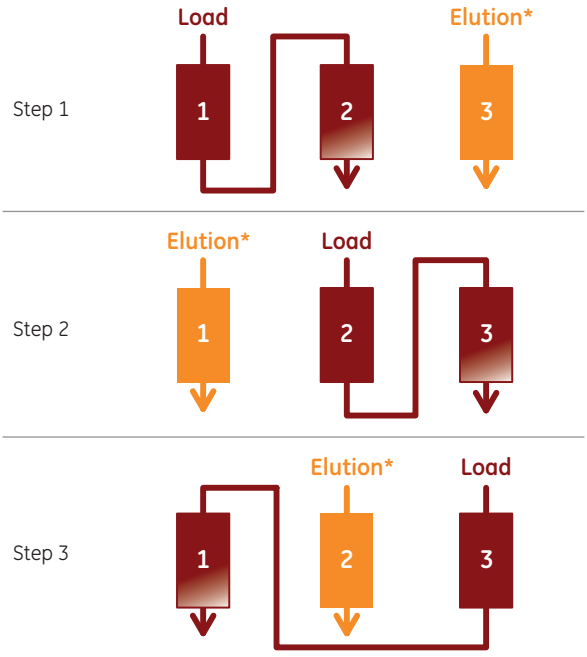

Fig 1. The principle of 3C PCC chromatography. Step 1: column 1 and 2 are loaded with clarified cell culture supernatant (red). Step 2: column 1 has reached the determined level of breakthrough and column 2 becomes the first column in the loading zone. In step 2, column 3 becomes the second column in the loading zone, while column 1 is subjected to wash, elution, strip, CIP, and re-equilibration (yellow). Step 3: column 2 has been loaded to the determined level of breakthrough and is disconnected from the loading zone and column 3 becomes the first column in the loading zone. In step 3, column 1 is now ready for the next cycle and becomes the second column in the loading zone. This procedure is repeated in a cyclic manner to achieve a continuous operation. * Elution phase in this figure includes wash, elution, strip, CIP, and re-equilibration.

Yield losses associated with the wash step (with product either in void or loosely bound to the chromatography medium) are minimized by the wash strategy described in Figure 2. The content of column 1 (saturated column) is washed and bound in column 3 (regenerated column) before column 3 is placed as second in the loading zone.

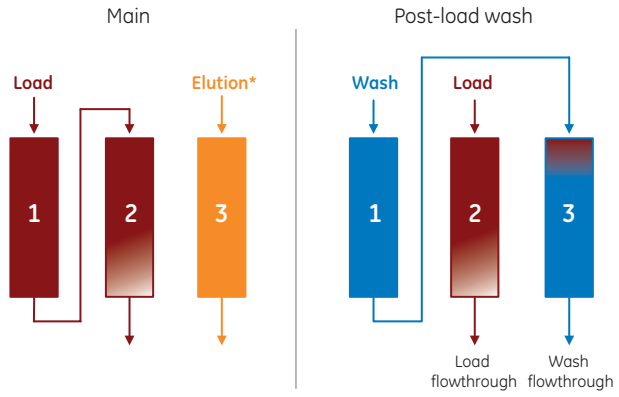

Fig 2. The wash strategy is used to avoid yield losses associated with the post-load wash step: column 1 (saturated column) is washed into column 3 (regenerated column) before column 3 is added into the loading zone. * Includes wash, elution, strip, CIP, re-equilibration.

Concept of STP

Downstream chromatography purification processes are traditionally performed as batch processes using hold-up tanks between the different unit operations. The separate chromatography steps, each optimized in terms of buffer substance, pH, and conductivity, often require intermediate conditioning steps. To remove or minimize the need of hold-up tanks and batch conditioning steps, an STP concept can be applied. STP is when two or more chromatography steps are connected in series, with in-line adjustment of process conditions between columns to ensure optimized performance of the next step. To omit the need for intermediate conditioning steps, elution conditions from the previous step can be selected to match the loading conditions for the subsequent step (4,5).

In this work, an ÄKTA pure system modified with an additional UV cell, mixer, and column valve was used.

Material and methods

Cell culture and harvest

The MAb used in this study was expressed in Chinese hamster ovary (CHO) cells. The cell culture was performed in fed-batch mode with daily feeds starting at a working volume of 150.5 L. Cells were harvested after 13 days at 200 L. Collection of supernatant was performed by centrifugation at 3000 × g. Finally, the supernatant was sterile filtered through ULTA™ Cap HC 0.2 µm filters into ReadyCircuit™ collection bags.

Chromatography media and columns

MabSelect SuRe LX protein A medium, packed in HiScreen™ columns (CV 4.7 mL), was used for the MAb capture step. Capto S ImpAct, a strong cation exchange medium, was used for the first polishing step. For the second polishing step, Capto adhere multimodal anion exchange medium was used. The polishing media were packed in Tricorn™ 5 columns.

Chromatographic methods and buffers

Capture step

The loading strategy for the 3C PCC setup was based on breakthrough studies using clarified cell culture supernatant. Breakthrough experiments were performed at different residence times. Based on the breakthrough curves, the dynamic binding capacity (DBC) was calculated as a function of residence time. The residence time and breakthrough levels were evaluated to maximize capacity utilization, while keeping the loading time longer than the time spent on the non-loading steps. In this case, a residence time of 5 min and a breakthrough level of 50% were selected. Loading was controlled using the dynamic control functionality of the ÄKTA pcc 75 system.

An overview of the process conditions for the MabSelect SuRe LX capture step is given in Table 1. For the experiments, clarified cell culture supernatant containing 4.5 g MAb/L was used. To assess robustness of the 3C PCC setup, 10 cycles were performed with the MabSelect SuRe LX capture step.

Table 1. Process conditions for the MabSelect SuRe LX capture step

Process step	Buffer	Column volumes (CV)	Residence time (min)
Equilibration	20 mM phosphate + 150 mM NaCl, pH 7.4	5	3.4
Sample load	67 g MAb/L medium	50% BT	5
Wash 1 (post-load wash)	20 mM phosphate + 150 mM NaCl, pH 7.4	1.5	6[†]
Wash 1	20 mM phosphate + 150 mM NaCl, pH 7.4	3.5	3.4
Wash 2	50 mM acetate, pH 6	1	3.4
Elution	50 mM acetate, pH 3.5	4	4
Strip	100 mM acetate, pH 2.9	2	3.4
CIP	100 mM NaOH	3	5
Re-equilibration	20 mM phosphate + 150 mM NaCl, pH 7.4	5	2

[†] Residence time for Wash 1 (post-load wash) should have been 5 min (same as for loading).

Polishing steps

Each polishing step was optimized individually to find conditions for optimal yield and purity in this study. For integration of the two polishing steps, column volume, flow rate, and intermediate sample conditioning were considered. The tendency of the used MAb to aggregate at a neutral pH narrowed the possible pH range and selection of suitable media for the polishing steps.

For the Capto S ImpAct B/E step, sample load was based on 70% of DBC at 10% breakthrough. The residence time was 5.4 min. Conditions for step elution were based on gradient runs.

For development of the Capto S ImpAct FT step, a design of experiments (DoE) approached was used. A central composite face-centered (CCF) design was set up using MODDE™ software v10 (Umetrics). Factors investigated are summarized in Table 2. The selected responses were, yield and aggregate content.

A DoE approach was also used for the development of the second polishing step using Capto adhere medium. A central composite face-centered (CCF) design was used and factors investigated are summarized in Table 3. In this case, the selected responses were yield and removal of aggregate and host cell protein (HCP).

The final conditions selected for the polishing steps are summarized in Tables 4 to 7.

Table 2. Factors investigated during development of the Capto S ImpAct FT step

Factor	Low	High
Load (mg MAb/mL medium)	50	170
Load NaCl concentration (mM)	200	250

Table 3. Factors investigated during development of the Capto adhere FT step

Factor	Low	High
Load (mg MAb/mL medium)	100	200
Load NaCl concentration (mM)	0	300
pH	5.5	6.5

Table 4. Process conditions for the Capto S ImpAct polishing step run in B/E mode

Process step	Buffer	CV	Residence time (min)
Equilibration	50 mM acetate + 50 mM NaCl, pH 5.0	3	5.4
Sample load	64 mg MAb/mL medium	-	5.4
Wash 1	50 mM acetate + 50 mM NaCl, pH 5.0	2	5.4
Wash 2	50 mM acetate + 125 mM NaCl, pH 5.0	2	5.4
Elution	50 mM acetate + 240 mM NaCl, pH 5.0	8	10.7
Strip	50 mM acetate + 500 mM NaCl, pH 5.0	2	5.4
CIP	1 M NaOH	3	5.4
Re-equilibration	50 mM acetate + 50 mM NaCl, pH 5.0	5	5.4

Table 5. Process conditions for the Capto S ImpAct polishing step run in FT mode

Process step	Buffer	CV	Residence time (min)
Equilibration	50 mM acetate + 230 mM NaCl, pH 5.0	3	5.4
Sample load	110 mg MAb/mL medium	-	7.7
Wash 1	50 mM acetate + 230 mM NaCl, pH 5.0	1	7.7
Wash 2	50 mM acetate + 230 mM NaCl, pH 5.0	2	5.4
Strip	50 mM acetate + 500 mM NaCl, pH 5.0	2	5.4
CIP	1 M NaOH	3	5.4
Re-equilibration	50 mM acetate + 230 mM NaCl, pH 5.0	5	5.4

Table 6. Process conditions for the Capto adhere polishing step run in FT mode (after Capto S ImpAct step run in B/E mode)

Process step	Buffer	CV	Residence time (min)
Equilibration	25 mM acetate + 25 mM phosphate + 120 mM NaCl, pH 6.2 or 6.3*	3	2.7
Sample load	128 mg MAb/mL medium	-	2.7
Wash	25 mM acetate + 25 mM phosphate + 120 mM NaCl, pH 6.2 or 6.3*	3	2.7
Strip	100 mM acetate buffer, pH 2.9	2	2.7
CIP	1 M NaOH	3	5
Re-equilibration	25 mM acetate + 25 mM phosphate + 120 mM NaCl, pH 6.2 or 6.3*	5	2.7

* Two different pH were tested (pH 6.2 and pH 6.3)

Table 7. Process conditions for the Capto adhere polishing step run in FT mode (after Capto S ImpAct step run in FT mode)

Process step	Buffer	CV	Residence time (min)
Equilibration	25 mM acetate + 25 mM phosphate + 115 mM NaCl, pH 6.2	3	2.7
Sample load	150 mg MAb/mL medium	-	2.7
Wash	25 mM acetate + 25 mM phosphate + 115 mM NaCl, pH 6.2	3	2.7
Strip	100 mM acetate buffer, pH 2.9	2	2.7
CIP	1 M NaOH	3	5
Re-equilibration	25 mM acetate + 25 mM phosphate + 115 mM NaCl, pH 6.2	5	2.7

Chromatography system

The ÄKTA pcc 75 chromatography system was used in the PCC setup. In the STP setup, the ÄKTA pure system modified with an additional UV cell, mixer, and column valve was used. Both systems are controlled by the UNICORN™ system control software.

Analytical methods

Elution pools from the chromatography runs were collected and analyzed for aggregate content by size exclusion chromatography (SEC) on a Superdex™ 200 Increase column. The peaks were integrated and the aggregate concentrations (in percent) were determined.

HCP content in the elution pools was analyzed using commercially available anti-CHO HCP antibodies (Cygnus Technologies Inc.) and Gyrolab™ workstation (Gyros AB).

Protein A content was determined using a commercially available ELISA kit (Repligen Corp.).

Results and discussion

Capture on MabSelect SuRe LX using PCC chromatography

The chromatogram in Figure 3 shows the loading step from each of the three columns of the 3C PCC setup over 10 cycles. The robustness of the capture step can be seen from the UV profiles. The UV curve post-column 1 shows identical height, length, and shape between loadings.

Alternatively, run consistency can be evaluated by calculating the loading time (time for a column at first position in the loading zone until desired level of breakthrough is reached). Table 8 shows that loading time was consistent between columns.

During these runs, 67 g MAb/L medium was loaded onto each column. This load can be compared with the possibility of loading 43 g MAb/L medium in batch mode (data not shown). A load of 43 g MAb/L medium corresponds to 70% of the DBC at 10% breakthrough (Q_{B10}) at 6 min residence time. The increased sample load in the 3C PCC setup corresponds to a 56% increase in chromatography medium capacity utilization.

The elution pool from each individual column and cycle was analyzed with respect to MAb amount, aggregate content, and HCP content (Fig 4). The performance was shown to be robust over the 10 cycles with regard to MAb purity and yield.

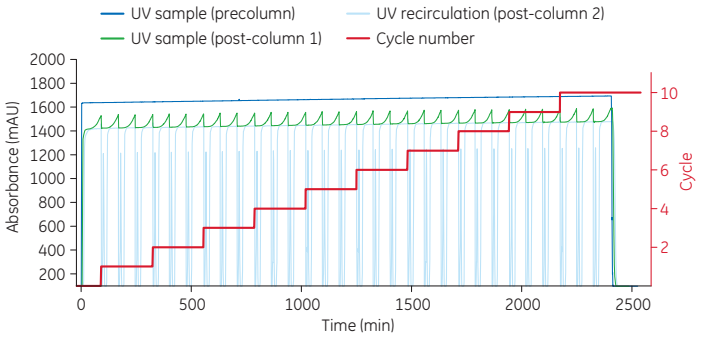

Fig 3. Chromatogram from each of the three columns in the 3C PCC setup during 10 cycles. Each cycle (red), constitutes one run per each of the three columns. The UV curve (dark blue) is the precolumn signal and displays the maximum absorbance. The UV curve represents the signal from both the background and the product. The background absorbance originates from both impurities and cell culture medium components. The post-column UV signal (green) represents the flowthrough of the first column in the loading zone. When all applied MAbs bind to the medium, the post-column UV signal will be equivalent to the absorbance level of the background. Eventually, product starts to break through. When breakthrough reaches a predefined absorbance level, the primary column in the loading zone is replaced. The absorbance after the second column in the loading zone (light blue) represents the background in the sample.

Table 8. Loading times (min) for each column and cycle

Column/ cycle	Loading time	Column/ cycle	Loading time	Column/ cycle	Loading time
C1/01	92*	C2/01	82*	C3/01	79
C1/02	81	C2/02	79	C3/02	84
C1/03	76	C2/03	77	C3/03	80
C1/04	80	C2/04	78	C3/04	78
C1/05	81	C2/05	78	C3/05	78
C1/06	81	C2/06	78	C3/06	78
C1/07	80	C2/07	78	C3/07	78
C1/08	80	C2/08	77	C3/08	78
C1/09	80	C2/09	77	C3/09	78
C1/10	80	C2/10	77	C3/10	77

* Steady-state not reached.

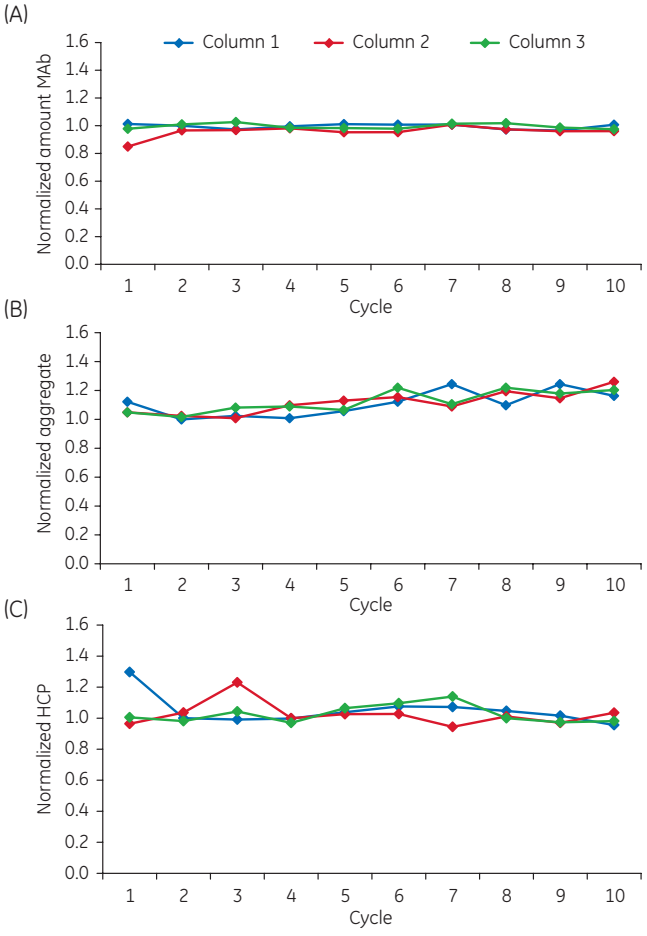

Fig 4. (A) Amount MAb, (B) aggregate content, and (C) HCP content in elution pool from the 3C PCC setup. The results are normalized against the elution pool from the first column in the second cycle (when steady state is reached).

Polishing on Capto S ImpAct and Capto adhere using STP

For Capto S ImpAct medium run in B/E mode, pH 5 was selected for the binding buffer. To increase binding capacity, 50 mM NaCl was included in the binding buffer. Sample load was 70% (64 g MAb/L medium) of Q_{B10} (92 g MAb/L medium). Step elution with 240 mM NaCl was developed based on a gradient run (data not shown).

For the development of the Capto S ImpAct step run in FT mode, a DoE approach was used. In Figure 5 shows a sweet spot analysis with the criteria > 90% yield and < 0.9% aggregates. Run conditions where both criteria were met were selected.

Also for the development of the Capto adhere step run in FT mode, a DoE approach was used. In Figure 6 shows a sweet spot analysis with the criteria > 85% yield, < 100 ppm HCP, and < 0.9% aggregates. Run conditions where all criteria were met were selected.

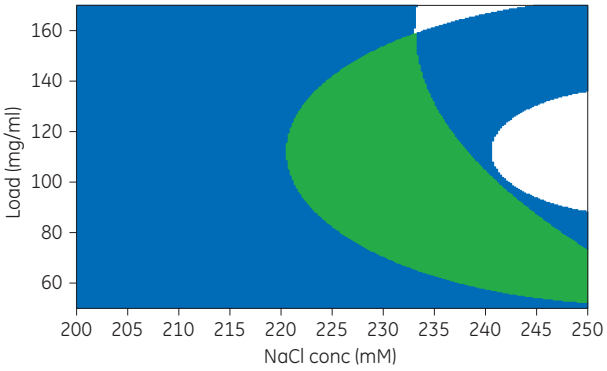

Fig 5. Sweet spot plot of the Capto S ImpAct FT step with the following predefined criteria: > 90% yield and < 0.9% aggregate content. Conditions where all criteria were met are represented by the green area. The running conditions chosen were 50 mM acetate + 230 mM NaCl, pH 5 as running buffer and a sample load of 110 g MAb/L medium. Blue area = one criterion fulfilled. White area = no criteria fulfilled.

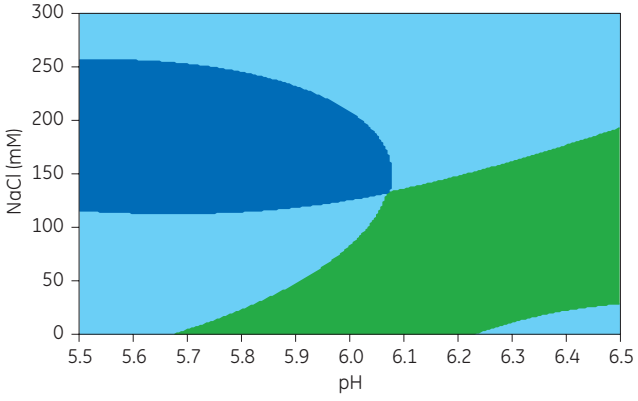

Fig 6. Sweet spot plot of the Capto adhere FT step with the following predefined criteria: > 85% yield, < 100 ppm HCP content, and < 0.9% aggregate content. Conditions where all three criteria were met are represented by the green area. The running conditions chosen were 25 mM acetate + 25 mM phosphate + 115 mM NaCl, pH 6.3 as running buffer and a sample load of 150 g MAb/L medium. Light blue area = two criteria fulfilled. Dark blue area = one criterion fulfilled.

For the MAb polishing steps, two different STP setups were evaluated, with the first polishing step run in either B/E or FT mode (Fig 7). An intermediate in-line conditioning step was included where pH was increased and the NaCl concentration lowered by dilution of the elution or FT pool with 50 mM phosphate + 24 mM NaOH.

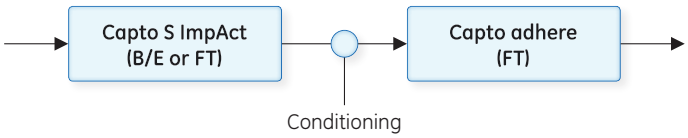

Fig 7. Setup of the MAb polishing steps.

A chromatogram from the STP setup combining Capto S ImpAct (B/E) and Capto adhere (FT) with an in-line conditioning step between columns is shown in Figure 8. To assess robustness, two STP experiments were performed with this approach and with the intermediate conditioning step slightly altered in pH between runs. Fractions were collected and analyzed for MAb yield and purity. As shown in Table 9, similar results were obtained in the parallel runs.

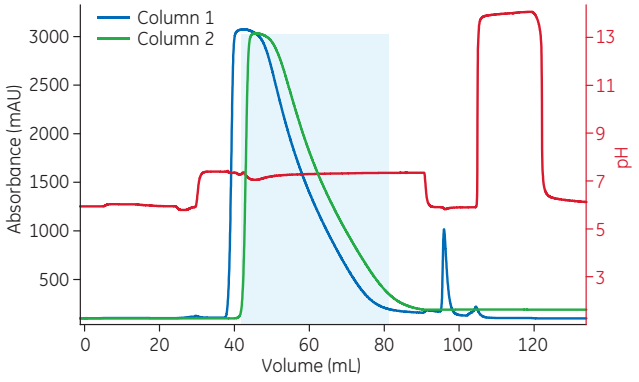

Fig 8. Chromatogram from the STP performed with Capto S ImpAct in B/E mode (obtained after in-line conditioning) followed by Capto adhere in FT mode. Flowthrough collected after the Capto adhere step is marked with a light blue area. Column 1: Capto S ImpAct (blue UV curve). Column 2: Capto adhere (green UV curve).

Table 10 shows analytical data from when both polishing steps were operated in FT mode. Although HCP content was shown to be slightly higher than with STP performed with the Capto S ImpAct step in B/E mode, high MAb purity and yield could be achieved also with this setup.

The STP results show similar yield and purity as can be expected when the individual unit operations are run in batch mode (data not shown).

Table 9. MAb yield and purity after STP on Capto S ImpAct (B/E) and Capto adhere (FT)

Process step	Yield over 2 steps (%)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
Capto S ImpAct (B/E)-Capto adhere (FT)	89	0.8	16	< 1
Capto S ImpAct (B/E)-Capto adhere (FT)	90	0.7	18	< 1

Note! Yields is shown as cumulated yield over the two steps, whereas aggregates, HCP and leached ligand are representing the final pool after both steps.

Table 10. MAb yield and purity after STP on Capto S ImpAct (FT) and Capto adhere (FT)

Process step	Yield over 2 steps (%)	Aggregates (%)	HCP (ppm)	Leached ligand (ppm)
Capto S ImpAct (FT)-Capto adhere (FT)	88	0.7	40	< 1

Note! Yields is shown as cumulated yield over the two steps, whereas aggregates, HCP and leached ligand are representing the final pool after both steps.

Conclusions

Here, results from a MAb purification using a continuous chromatography approach are presented. Protein A capture was conducted using PCC chromatography with the goal to increase capacity utilization. Corresponding to 30 sample loads, 10 cycles were performed on a 3C PCC setup. The performance of each column was evaluated with regard to MAb yield and purity in terms of aggregate and HCP removal. The results show consistent performance for the three columns over all cycles. The observed product quality and yield for the capture step were similar to what can be obtained in an equivalent batch column setup. Compared with a batch run, however, the capacity utilization was increased by 56% using the 3C PCC setup.

MAb polishing was performed using an STP setup comprising two serially connected columns with an intermediate in-line conditioning step. The results show MAb yield and purity similar to what is obtained in a traditional setup performed in batch runs.

This work shows how continuous chromatography can be used to increase utilization of the chromatography medium capacity and eliminate the need for intermediate hold-up tanks, while reducing equipment footprint.

References

1. Mahajan, E., George, A., Wolk, B. Improving affinity chromatography resin efficiency using semi-continuous chromatography. *Journal of chromatography A*, **1227**, 154-162 (2012).
2. Pollock, J., Bolton, G., Coffman, J., Ho, S.V., Bracewell, D.G., Farid, S.S., Optimising the design and operation of semi-continuous affinity chromatography for clinical and commercial manufacture. *Journal of Chromatography A*, **1284**, 17-27 (2013).
3. Application note: The use of dynamic control in periodic counter-current chromatography. GE Healthcare, 29169455 , Edition AA (2015).
4. Kumar, V., Rathore, A.S. Two stage chromatographic separation of aggregates for monoclonal antibody therapeutics, *Journal of chromatography A*, **1368**, 155-162 (2014).
5. Landric-Burtin, L. Simplicity is the key. Continuous purification of monoclonal antibodies. Presented at *Integrated Continuous Biomanufacturing Conference*, Barcelona, (2013).

Ordering information

Product	Size	Product code
MabSelect SuRe LX	25 mL	17547401
HiScreen MabSelect SuRe LX	4.7 mL	17547415
Capto S ImpAct	25 mL	17371701
Capto adhere	25 mL	17544410
Tricorn columns	5/50	28406409
Tricorn columns	5/150	28406411
Superdex 200 Increase 10/300	24 mL	28990944

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