Technical and process economical aspects of using Capto™ Q and ReadyToProcess™ Adsorber Q in mAb polishing

Monoclonal antibodies (mAbs) are typically purified using a platform approach, including a protein A-based capture step followed by one or two polishing steps. This application note compares the use of membrane chromatography and resin chromatography in the final polishing step in a three-step mAb purification process. Optimal conditions, with regard to pH and conductivity, were determined in PreDictor™ 96-well plate experiments. Selected conditions were verified in column or membrane capsule experiments. The results show that the ReadyToProcess Adsorber Q membrane and Capto Q resin are equally efficient in removing final impurities in a mAb purification process. Scenarios for when each of these product options is more beneficial were identified in a process economy simulation.

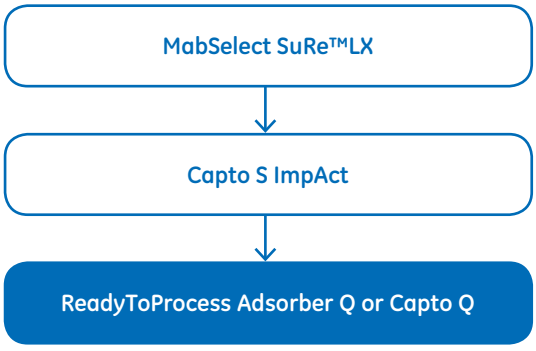

Fig 1. Classical three-step mAb purification process, using MabSelect SuRe LX protein A resin for capture, Capto S ImpAct cation exchange resin for intermediate purification, and either ReadyToProcess Adsorber Q membrane or Capto Q resin for final polishing by anion exchange chromatography.

Introduction

Increasing product titers in upstream cell culture processes pose challenges to downstream purification processes. For efficient operations, downstream purification needs to be able to handle high product titers at short process time. Compared with conventional chromatography, using stainless steel columns, the use of ReadyToProcess Adsorber membranes can help reduce capital investment, consumption of buffers and other process liquids for cleaning and storage, as well as process time, for example, by minimizing the need for cleaning and validation operations and by allowing for higher flow rates.

While sometimes perceived as costly, these ready-to-use products minimize the need for hardware qualification and cleaning validation, which can be rather costly and time-consuming for biomanufacturers. By minimizing the need for cleaning operations, thereby reducing the changeover time between campaigns, ReadyToProcess Adsorber membranes allow for more batches to be produced per year, improving facility utilization and thus the profit opportunity. Compared with facilities using stainless steel columns, the use of disposable ReadyToProcess Adsorber membranes minimizes the number of non-value-adding steps in process development, such as cleaning validation and lifetime studies. In a bioprocessing setup, ReadyToProcess Adsorber membranes enable elimination of typical bottlenecks, such as facility fit, processing time, cleaning procedures, and overall changeover times.

At larger scales, however, conventional chromatography, using stainless steel equipment, can still be a beneficial alternative. Here, we compare the performance of the disposable ReadyToProcess Adsorber Q membrane capsule with Capto Q resin packed in conventional chromatography columns when used in the final polishing step of a three-step mAb purification process (Fig 1). To identify scenarios for beneficial use of each of these product alternatives, a process economy simulation was conducted for two different process scales (500 and 2000 L).

Table 1. Running conditions used in column experiments

Parameters	Capto Q column	ReadyToProcess Adsorber Q
Volume	2 mL	1 mL
Flow rate	2.33 mL/min	20 mL/min
Precleaning (1 M NaOH)	10 CV (pause for 30 min)	30 MV
NaCl strip (1 M NaCl)	10 CV	20 MV
Equilibration (20 mM phosphate buffer, pH 7.5)	40 CV	50 MV
Sample load	200 mg mAb/mL resin	2000 mg mAb/mL membrane
Wash (20 mM phosphate buffer, pH 7.5)	20 CV	50 MV
NaCl strip (1 M NaCl)	10 CV	N/A (unless reused)
Post-cleaning (1 M NaOH)	10 CV (pause for 30 min)	N/A (unless reused)
Equilibration (20 mM phosphate buffer, pH 7.5)	40 CV	N/A (unless reused)

CV = column volumes, MV = membrane volumes

Materials and methods

Sample preparation

For this study, mAb was expressed in Chinese hamster ovary (CHO) cells. Cell culture clarification was performed by centrifugation and filtration, using ULTA™ HC disposable filter capsules. Using standard operating conditions, mAb capture was performed on MabSelect SuRe LX resin, the eluate was filtered using an ULTA HC disposable filter capsule, and further subjected to intermediate purification on Capto S ImpAct resin. Before final polishing, the product was buffer exchanged on Sephadex™ G-25 resin.

Determination of process conditions

Optimal pH and conductivity for maximized host cell protein (HCP) reduction were determined in 96-well plate experiments, using either PreDictor Capto Q (2 µL/well) or PreDictor ReadyToProcess Adsorber Q (19 µL/well) plates. In 11 mM phosphate buffer, pH was varied between 6.0 and 8.0 in intervals of 0.4 units, and NaCl concentration was varied between 0 and 80 mM in intervals of 26.67 mM. Sample load was 2250 mg mAb/mL Capto Q resin or 395 mg mAb/mL ReadyToProcess Adsorber Q membrane. The large difference in load was due to loading maximum volume in each well, which was 500 µL for ReadyToProcess Adsorber Q and 300 µL for Capto Q, with the aim of loading as much HCP per well as possible. Following specific experimental procedures for the different PreDictor plates resulted in the use of different contact times for the resin and membrane.

Verification of process conditions

Optimal process conditions, determined in PreDictor screening experiments, were verified in capsule and column experiments, using either the ReadyToProcess Adsorber Q 1 mL capsule or a 2 mL Tricorn™ column packed with Capto Q resin to a 10 cm bed height. Both formats were run in flow-through mode. Running conditions are listed in Table 1.

Analytical methods

The mAb concentration was determined spectrophotometrically at 280 nm (mAb coextinction coefficient = 1.06). HCP content was measured by an ELISA method using Gyrolab™ Workstation LIF (Gyros AB) and antibodies from Cygnus Technologies. Levels of host cell DNA (hcDNA) were determined by an in-house qPCR method, using primers and probes as described previously (1). Samples were automatically prepared using a MagMax™ Express 96-deepwell magnetic particle processor and PrepSEQ™ Residual DNA Sample Prep kit. Real-time PCR was performed using the StepOnePlus™ system, and by using the StepOne™ software for evaluation.

All experiments were performed twice and mAb content was determined in each duplicate sample. For HCP and hcDNA analyses, the duplicate samples were pooled.

Process economy simulation

Based on a three-step mAb purification, the process economy simulation compares the final polishing step, using either ReadyToProcess Adsorber Q capsules or Capto Q packed in conventional acrylic/stainless steel columns. Cell culture scales of 500 and 2000 L, with product titers of 5 mg mAb/mL, were used as starting material.

Major assumptions made in the comparison:

- Sample volume: 10% of cultivation volume (i.e., 50 or 200 L)
- Sample recovery: 80% of start amount in cell culture (i.e., initially 5 mg mAb/mL)
- Sample load (with a typical HCP level of a few hundred ppm) (2)
  - ReadyToProcess Adsorber Q: 2000 g mAb/L membrane
  - Capto Q: 200 g mAb/L resin
- Maximum process time: 150 min

Costs included in the comparison:

- Buffers and other process liquids
- Capto Q lifetime assay
- Column packing and storage
- Cost for manufacturing site and labor
- Cleaning validation
- ReadyToProcess Adsorber Q
- Capto Q resin
- Stainless steel column

Cost information is based on GE Healthcare's list prices, in-house experience, as well as an external source (3).

Costs not included in the comparison:

- Chromatography system capital investment (infrastructure assumed to be in place)
- Labor costs associated with process preparations, for example, connection of column/capsule.

For Capto Q, the fixed costs are related to the resin and resin lifetime assay, as well as to column hardware, packing, and storage- and cleaning validation. Reoccurring batch costs are related to the buffers and corresponding manufacturing suite, as well as to labor during process time.

For ReadyToProcess Adsorber Q, the device is used entirely as a disposable, with no fixed costs, but only reoccurring costs of the membrane adsorber and costs related to the buffers and corresponding manufacturing suite, as well as to labor during process time.

Methods that were compared are summarized in Table 2. Flow velocity for Capto Q was set to 700 cm/h. The turnover rate was set to 15 membrane volumes for ReadyToProcess Adsorber Q. These set-points correspond to the actual values used during the column verification experiments described in this application note.

Table 2. Chromatography methods included in the process economy simulation

Method phases	Capto Q	ReadyToProcess Adsorber Q
Equilibration	10 CV	20 MV
Sample load	200 mg mAb/mL resin	2000 mg mAb/mL membrane
Wash	5 CV	5 MV
Strip	2 CV	N/A
CIP	3 CV	N/A
Re-equilibration	5 CV	N/A

CIP = cleaning in place, CV = column volumes, MV = membrane volumes

Results

For this study, comparing the use of ReadyToProcess Adsorber Q membranes and Capto Q resin, mAb was purified from cell culture supernatant. As summarized in Table 3, levels of HCP and hcDNA were significantly reduced already in the capture and intermediate purification steps (which is typical after MabSelect SuRe LX and Capto S ImpAct), resulting in low impurity level in the sample prepared for the final polishing step. Therefore, only HCP levels were continued to be monitored in this study, while hcDNA levels were not further determined.

Table 3. Process for mAb sample preparation from cell culture supernatant

Process step	mAb conc. (mg/mL)	HCP (ng/mL)	HCP (ppm)	hcDNA (ng/mL)	hcDNA (ppb)
Clarified cell harvest	2.4	2 385 222	990 000	80 959	33 700 000
Capture (MabSelect SuRe LX)	17.26	32 566	1887	10	579
Filtration on ULTA HC filter capsule	17.47	9932	569	3	172
Intermediate purification (Capto S ImpAct)	21.17	4665	220	n.d.	N/A
Desalting (Sephadex G-25)	16.66	2402	144	n.d.	N/A

N/A = not applicable, n.d. = not determined

PreDictor plate experiments

HCP reduction was optimized in PreDictor plate experiments. To ensure maximum load of HCP, PreDictor plates with as low resin or membrane volume as possible were selected. HCP content in the starting material was 144 ppm.

For Capto Q experiments, sample load was 0.32 mg HCP/mL resin. For the ReadyToProcess Adsorber Q experiments, sample load was 0.057 mg HCP/mL membrane. As shown in Figure 2, greatest HCP reduction was achieved at high pH and low conductivity for both product alternatives. With membrane chromatography, an overall greater HCP reduction could be achieved already at a lower pH and with some salt present in the buffer.

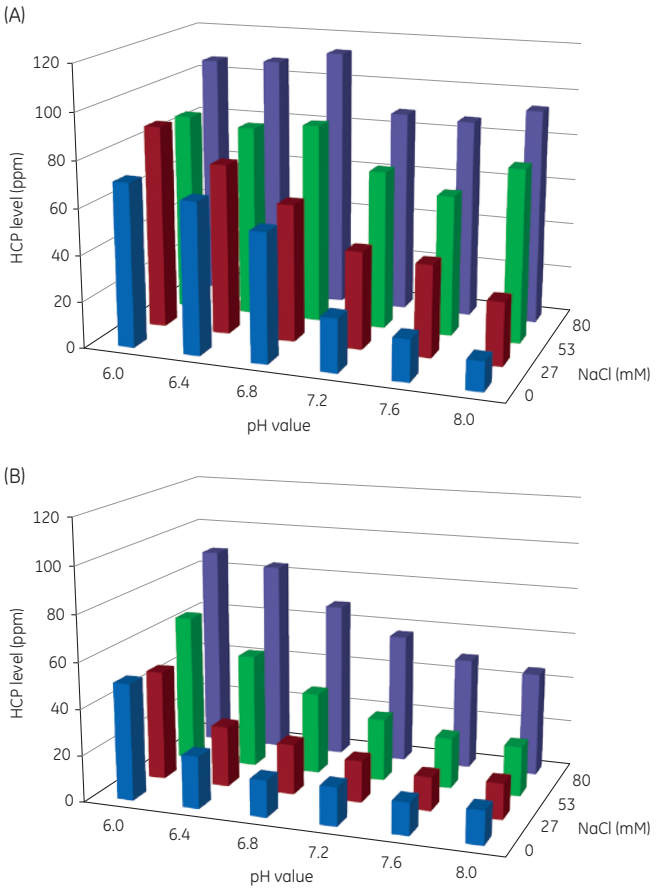

Fig 2. HCP reduction in (A) PreDictor Capto Q and (B) PreDictor ReadyToProcess Adsorber Q 96-well plate experiments (amount of HCP at start was 144 ppm).

Column verification

Optimized process conditions for maximized HCP reduction, as determined in PreDictor experiments, were verified in column or capsule experiments, using 20 mM phosphate buffer, pH 7.5 as running buffer. The starting material (prepared as for the PreDictor experiments) contained 180 ppm HCP. The flowthrough was fractioned, and HCP content in fractions 2, 6, and 10 is displayed in Figure 3. As expected, HCP reduction was comparable with the results obtained in the PreDictor plate experiments.

The column verification experiments indicated a somewhat greater HCP reduction with Capto Q compared with ReadyToProcess Adsorber Q. For the PreDictor plate experiments, the opposite was shown, with a larger HCP reduction using the membrane. However, the difference between the results from PreDictor plate and column verification experiments is small and can be attributed to variation in the measurement method. The difference in sample load (see Section Materials and methods) as well as the different contact times used (due to different procedures between plate and column experiments) can also contribute to the observed difference in HCP reduction between experiments. The mAb recovery for the column experiments was found to be in the range of 97%–100% for both the resin and membrane.

As shown from both plate and column experiments, both product alternatives offered good HCP reduction. However, while ReadyToProcess Adsorber Q capsules can be operated at high flow rates, Capto Q exhibits a larger binding capacity for HCP.

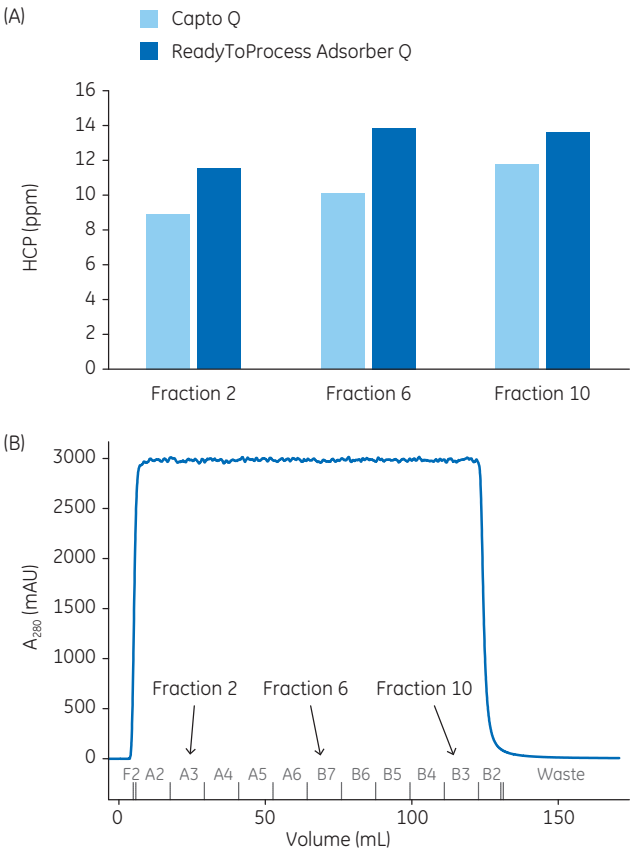

Fig 3. (A) HCP content in selected fractions from the column verification experiments (amount of HCP at start was 180 ppm). (B) Chromatogram from the ReadyToProcess Adsorber Q run, showing fractions analyzed for HCP content (the Capto Q run resulted in a similar chromatogram).

Process economy simulation

500 L process scale

At 500 L scale, a column with an inner diameter of 30 cm and packed with 10.6 L Capto Q to a 15 cm bed height was sufficient to fulfill the requirements for sample load and process time. For ReadyToProcess Adsorber Q, a 1.2 L capsule was used to meet the same requirements. The results displayed in Figure 4 show that conventional resin chromatography exhibits a higher initial cost due to the fixed costs associated with this alternative. Up to 70 batches can be produced with membrane chromatography before the use of resin becomes the more beneficial alternative.

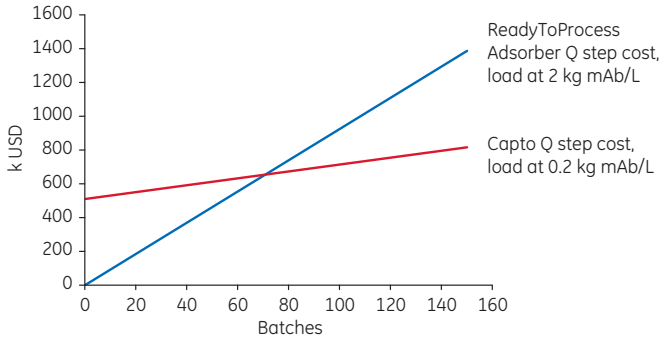

Fig 4. Accumulated mAb manufacturing costs for the final polishing step (500 L process scale). For Capto Q, costs for labor, buffer/process liquids, resin, column hardware, resin packing and lifetime study, as well as cleaning and storage of the packed column are included. For ReadyToProcess Adsorber Q, costs for membrane, labor, and buffer/process liquids are included.

2000 L process scale

At 2000 L scale, a column with an inner diameter of 60 cm and packed with 42.4 L Capto Q to a 15 cm bed height was selected to fulfill the requirements for sample load and process time. For ReadyToProcess Adsorber Q, a 5 L capsule was selected to meet the same requirements. As shown in Figure 5, membrane chromatography is a cost-efficient alternative also for this scale. However, at this scale, only 19 batches are required before conventional resin chromatography becomes the more beneficial alternative.

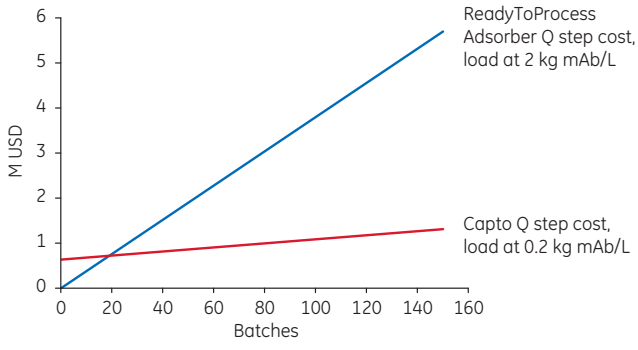

Fig 5. Accumulated mAb manufacturing costs for the final polishing step (2000 L process scale). For Capto Q, costs for labor, buffer/process liquids, resin, column hardware, resin packing and lifetime study, as well as cleaning and storage of the packed column are included. For ReadyToProcess Adsorber Q, costs for membrane, labor, and buffer/process liquids are included.

Conclusions

In this work, the performance of the ReadyToProcess Adsorber Q membrane was compared with that of Capto Q resin when used for HCP reduction in the final polishing step of a three-step mAb purification scheme. Under the conditions used, a similarly efficient HCP reduction was achieved with membrane chromatography as with conventional resin chromatography. Comparable results were obtained between the product alternatives under the various conditions tested in PreDictor plate experiments for optimization of HCP reduction. For selected conditions, the results were confirmed in larger scale column or capsule experiments. However, while the membrane can be used with higher flow rates, the resin allows for greater absolute reduction of HCP level.

In a process economy simulation, the use of ReadyToProcess Adsorber Q and Capto Q in 500 and 2000 L scales was compared. The results show that the choice of product depends on scale and number of batches. In the smaller scale, membrane chromatography is the more cost-efficient option for up to 70 batches. In the larger scale, conventional resin chromatography becomes the more cost-efficient alternative already at 19 production batches. However, as ReadyToProcess Adsorber Q membrane capsules require no cleaning validation, and thus allowing for more annual production batches, this alternative generates a greater profit opportunity than conventional chromatography using stainless steel equipment.

ReadyToProcess Adsorber membranes are intended and validated for single use to avoid carryover as well as tedious and costly cleaning and cleaning validation procedures, although technically possible to reuse after cleaning in place depending on application, character of sample, and process. Additional validation steps will be needed to ensure effective cleaning procedure as well as constant binding capacity and flow rate after each cycle.

A third alternative, not included in this study, is Capto Q resin provided prepacked in ReadyToProcess columns. While offering the higher binding capacity of Capto Q, the ReadyToProcess Capto Q column, operated through the ÄKTA™ ready chromatography system equipped with a single-use flow path, provides a similarly disposable alternative as ReadyToProcess Adsorber Q when operated through a pump or system that allows the use of a single-use flow path. As for ReadyToProcess Adsorber Q, the use of ReadyToProcess Capto Q contributes to reduced costs for hardware, column packing as well as cleaning and storage validation, and resin lifetime study. However, implications for using ReadyToProcess Adsorber Q or ReadyToProcess Capto Q need to be assessed in process economy calculations.

Disclaimer

The results and conclusions presented in this application note are valid for this specific study. Other study conditions and assumptions could have significant impact on the outcome. The overall finding in this study is that the ReadyToProcess Adsorber Q membrane and Capto Q resin are equally efficient in reducing HCP levels in the final polishing step of a three-step purification process for the used mAb. With initially higher impurity levels in the starting material, the use of Capto Q resin, with its higher binding capacity, could prove to be more beneficial for impurity reduction than the ReadyToProcess Adsorber Q membrane. Scenarios for when the use of which product alternative is more beneficial depend on process economy and overall impurity levels. Capto Q and ReadyToProcess Adsorber Q are therefore complementing each other, and product recommendations require detailed insight into the individual process.

References

1. Hu, B., Sellers, J., Kupec, J., Ngo, W., Fenton, S., Ynag, T.Y., Grebanier, A. Optimization and validation of DNA extraction and real-time PCR assay for the quantitative measurement of residual host cell DNA in biopharmaceutical products. *J Pharm Biomed Anal* **88**, 92–95 (2014).
2. Anticipated post-launch change: other AEX formats in *A-Mab: a case study in bioprocess development*, Version 2.1. CMC Biotech Working group, pp 151–152 (2009).
3. Zhou, J.X. and Tressel, T. Basic concepts in Q membrane chromatography for large-scale antibody production. *Biotechnol Prog* **22**, 341–349 (2006).

Ordering information

Product	Description	Product code
Capto Q	1 L	17547803
ReadyToProcess Adsorber Q nano 4mm	1 mL	17372102
PreDictor Capto Q	2 µL 96-well plate	28925773
PreDictor ReadyToProcess Adsorber Q	19 µL 96-well plate	17372119

Related literature

Instruction: PreDictor ReadyToProcess Adsorber plates	29152709
Instruction: PreDictor plates	28925834

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