Purification of a monoclonal antibody using ReadyToProcess™ columns

BIOPROCESS™ PREPACKED COLUMNS

Key words: Monoclonal antibodies, Process-scale chromatography, Prepacked columns

Abstract

To reduce costs and shorten time to market, the use of plug-and-play technology is increasing in process development as well as in later-stage production. ReadyToProcess™ columns are prepacked, prequalified, and presanitized columns ready for direct use.

In this study, the performance of ReadyToProcess™ columns prepacked with MabSelect SuRe™, Capto™ Q, and Capto™ adhere resins was compared with small-scale XK16/40 (XK) columns packed with the same resins for the purification of a monoclonal antibody (mAb) in a three-step process in parallel experiments. Yield and contaminant levels were practically identical during all steps, demonstrating the comparable performance of the column types and that the process is scalable. In addition, ReadyToProcess™ columns can be used for repeated runs with retained performance.

Introduction

The increasing demand for mAbs as biopharmaceuticals has promoted the development of efficient processes for cell culturing, as well as for purification. Plug-and-play units make several time-consuming steps redundant, and therefore shorten time to market. Such solutions also reduce the risk of cross-contamination significantly.

ReadyToProcess™ columns are prepacked, prequalified, and presanitized process chromatography columns, suited for purification of biopharmaceuticals (e.g., proteins, vaccines, plasmids, viruses) for clinical phase I and II studies. The columns are ready for use and the design makes them easy to connect to chromatography systems and to dispose of after completed production. ReadyToProcess™ columns are available with a range of BioProcess™ resins in several sizes.

In this study, of ReadyToProcess™ columns was compared with an established small-scale format. A mAb was purified from cell culture supernatant using a three-step, generally applicable process consisting of MabSelect SuRe™, Capto™ Q, and Capto™ adhere. The BioProcess™ resins in the ReadyToProcess™ columns are the same as those used in conventional process chromatography, thus allowing the use of a fully flexible mode in early production while keeping a conventional re-use option for later large-scale manufacturing open.

Material and methods

ReadyToProcess™ columns (2.5 L column volume [CV]) and XK16/40 columns (40 mL CV) packed with the same resins and having the same bed height (20 cm) were used to compare the performance of the column types and to demonstrate scalability.

The three-step purification strategy involved capture using MabSelect SuRe™, an affinity resin with an alkali-tolerant protein A-derived ligand. Further, interresinste purification using ion exchange was employed with Capto™ Q followed by a final polishing step of the mAb with Capto™ adhere. The columns were connected to ÄKTAexplorer™ 100 (XK columns) and ÄKTAprocess™ (ReadyToProcess™ columns) chromatography systems. UNICORN™ software was used for control and evaluation.

By using a platform approach, the development time and effort was kept to a minimum and the development work was concentrated on the third, polishing step, where the multimodal anion exchanger Capto™ adhere was used.

MabSelect SuRe™ step

The feed consisted of filtered CHO cell culture supernatant containing 2.7 mg mAb/mL. Sample volumes corresponding to 25 mg mAb/mL bed volume were applied to the XK 16/40 and ReadyToProcess™ 2.5 L column packed with MabSelect SuRe™. Five cycles, each including cleaning-in-place (CIP) with 0.5 M NaOH, were run on each column, and the eluates were collected using an UV watch function.

mAb purification at large scale typically contains a virus inactivation step at low pH after the protein A capture step, taking advantage of the low pH of the collected eluate. This step was omitted in this study. To match the buffer conditions of the equilibration buffer in the subsequent Capto™ Q step, pH of the collected eluates was imresinstely adjusted to 7.6.

Capto™ Q step

The pH-adjusted eluates from the five MabSelect SuRe™ runs werepooled and applied to the Capto™ Q column in flowthrough mode.The flowthrough and part of the washing solution were collectedand prepared for the Capto™ adhere step by adjusting the conductivity and pH to match the conditions of the equilibration buffer in the Capto™ adhere step.

Capto™ adhere step

All material from the Capto™ Q run was applied to Capto™ adhere in flowthrough mode. The flowthrough and washing solution were collected.

Analytical methods

Samples were withdrawn for analysis at each stage of the purification process. The amount of dimer and aggregates inthe samples was determined by gel filtration on a Superdex™ 200 10/300 GL column. Host cell protein (HCP) concentration was determined using the CHO-CM HCP ELISA kit (CM015, Cygnus Technologies). The concentration of leached MabSelect SuRe™ ligand was determined by a protein A ELISA method using purified ligand for the ELISA standard curve, essentially as described by Steindl *et al*. (1). The analyses were not optimized for this particular feed and mAb.

Table 1. Process yield

	Volume (mL)		mAb concentration (mg/mL)		Total mAb (mg)		Step yield (%)		Overall yield (%)	
Sample	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**
Filtered feed[1]	1880	116000	2.7	2.7	5080	313200	100	100	100	100
MabSelect SuRe™ eluate[1]	340	25230	14.1	11.5	4790	290100	94	93	94	93
Capto™ Q flowthrough	403	29500	11.6	10.0	4680	295000	98	102	93	94
Capto™ adhere flowthrough	767	51200	5.7	5.5	4370	281600	95	94	88	88

[1] Volumes and amounts pooled from the five MabSelect SuRe™ runs

Table 2. Contaminant levels throughout the purification process

	HCP concentration				Protein A concentration				Dimer/aggregates content	
	(ng/mL)		(ppm)		(ng/mL)		(ppm)		(%)	
Sample	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**
Filtered feed	63300	63300	23444	23444	N/A	N/A	N/A	N/A	N/A	N/A
MabSelect SuRe™ eluate	689	704	49	61	11.5	8.0	0.8	0.7	4.1	3.8
Capto™ Q flowthrough	114	133	10	13	5.5	n.q.[1]	0.5	n.q.[1]	3.1	2.9
Capto™ adhere flowthrough	52	76	9	14	n.q.[1]	n.q.[1]	n.q.[1]	n.q.[1]	0.3	0.2

[1] Not quantifiable; limit of quantitation is 5 ng/mL.
RTP = ReadyToProcess™ column

Column:	MabSelect SuRe™, 40 mL in XK16/40 (A) and RTP MabSelect SuRe™ 2.5 (CV 2.5 l, B), bed height 20 cm
Equilibration buffer:	0.02 M sodium phosphate, 0.15 M sodium chloride, pH 7.4 (1.2 CV)
Sample:	Filtered CHO cell culture supernatant
Sample load:	25 mg mAb/mL resin (approximately 9.3 CV)
Wash:	1) Equilibration buffer (3 CV) 2) 0.025 M sodium phosphate, 5% isopropanol, 0.5 M sodium chloride, pH 7.0 (2 CV), 3) Equilibration buffer (2 CV), 4) 0.01 M sodium phosphate 0.01 M sodium citrate, pH 7.6 (2 CV)
Elution:	0.01 M sodium phosphate, 0.01 M sodium citrate, pH 3.5 (3 CV)
pH adjustment:	Equilibration buffer (1 CV)
CIP:	0.5 M NaOH (3 CV)
Re-equilibration:	Equilibration buffer (3 CV)
Liquid velocity:	400 cm/h
System:	ÄKTAexplorer™ 100 (A), ÄKTAprocess™ (B)

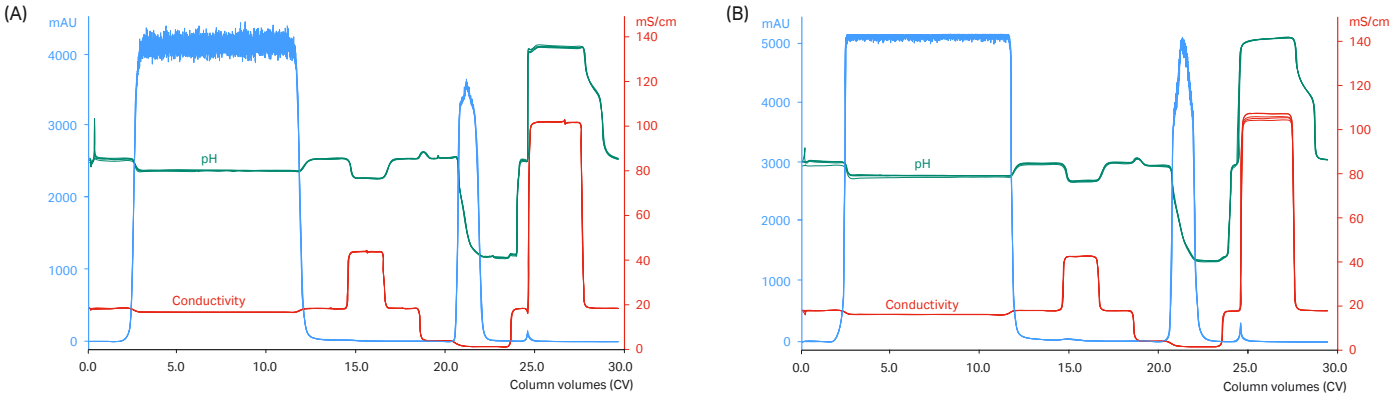

Fig 1. Comparison of the capture of a mAb using **(A)** an XK 16/40 column packed with MabSelect SuRe™ and **(B)** a prepacked ReadyToProcess™ 2.5 L column packed with MabSelect SuRe™. Each chromatogram is an overlay of absorbance, conductivity, and pH curves from five identical runs.

Results

A three-step monoclonal antibody purification was performed in parallel at two different scales. The overall yield was 88% for both processes (Table 1), achieving contaminant levels acceptable for formulation.

The three-step purification process is characterized by an overall good yield, low ligand leakage from MabSelect SuRe™, and efficient contaminant and dimer/aggregate removal. It should be emphasized that the process development in this study was limited, since the conditions for the two first steps, MabSelect SuRe™ and Capto™ Q, are more or less generic, while the final Capto™ adhere step required some evaluation of operating conditions. The aim of this study was not to evaluate the purification process as such, but to study the performance of the ReadyToProcess™ columns in a purification process, and compare the results to another already established column format (XK). Therefore, details on purification levels are not discussed below.

A summary of contaminant reduction data for the whole process is given in Table 2. With limited optimization, acceptable levels of impurity were obtained after three steps.

Table 3. Yield in eluates from XK columns packed with MabSelect SuRe™ and ReadyToProcess™ 2.5 L column packed with MabSelect SuRe™, run 1-5

	Volume (mL)		mAb concentration (mg/mL)		Total mAb (mg)		Yield (%)	
Sample	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**
Filtered feed	376	23200	2.7	2.7	1020	62640	100	100
Eluate run 1	67	5040	14.1	11.7	940	58970	93	94
Eluate run 2	68	5030	14.3	11.7	970	58850	96	94
Eluate run 3	68	5070	13.8	11.6	940	58810	92	94
Eluate run 4	69	5060	13.6	11.6	940	58700	92	94
Eluate run 5	69	5030	13.5	11.6	930	58350	92	93

Table 4. Contaminant levels in eluates from XK columns packed with MabSelect SuRe™ and ReadyToProcess™ 2.5 L column packed with MabSelect SuRe™ 2.5, run 1-5

	HCP concentration (ppm)		Protein A concentration (ppm)		Dimer/aggregates content (%)	
Sample	**XK**	**RTP**	**XK**	**RTP**	**XK**	**RTP**
Filtered feed	23444	23444	N/A	N/A	N/A	N/A
Eluate run 1	52	64	2.5	1.6	3.6	3.4
Eluate run 2	51	63	0.6	0.6	3.9	3.2
Eluate run 3	53	61	0.4	0.4	4.1	3.6
Eluate run 4	54	63	0.4	0.4	3.9	3.4
Eluate run 5	58	66	0.4	0.4	3.6	3.6

MabSelect SuRe™ step

Both MabSelect SuRe™ columns were run five times each to investigate the effects of repeated runs on column performance, as well as to gather enough material for subsequent chromatography steps. The chromatograms obtained were similar (Fig 1). The uniform performance was confirmed by the analytical results.

Yields were stable during all cycles (Table 3), and the contaminant levels were comparable (Table 4). The HCP level was efficiently reduced, and the ligand leakage low, which is characteristic for MabSelect SuRe™. The higher level of ligand leakage in the first cycle, which was detected on both column types (Table 4), is typical for protein A-based chromatography resins. As a result of differences in scale, chromatography systems, and UV detectors, the relative eluate volumes measured in CV, were slightly different in the XK and ReadyToProcess™ runs. Each of the five eluates from the XK runs had volumes corresponding to 1.7 CV, while the five eluates from the ReadyToProcess™ runs had volumes corresponding to 2.0 CV. Therefore, the sample volumes in the subsequent Capto™ Q and Capto™ adhere steps were smaller for the XK runs compared to the ReadyToProcess™ runs. This difference becomes apparent when comparing the XK and the ReadyToProcess™ chromatograms from the Capto™ Q and Capto™ adhere runs (Fig 2 and 3).

Capto™ Q step

A comparison between the Capto™ Q run on the XK column and the ReadyToProcess™ column is shown in Figure 2. The mAb-containing flowthrough and part of the wash were collected. Again, the comparable performance of the XK and ReadyToProcess™ columns was confirmed by the analytical results. The Capto™ Q step was characterized by high yield (Table 1), reduction of HCP, and some reduction of leached ligand and dimer/aggregates (Table 2).

Capto™ adhere step

A comparison between the Capto™ adhere run on the XK column and the ReadyToProcess™ column is shown in Figure 3. The mAb-containing flowthrough and all of the wash were collected. Again, the comparable performance of the XK and ReadyToProcess™ columns was confirmed by the analytical results. The Capto™ adhere step had a high yield and efficiently reduced the amount of dimers and aggregates in this study (Table 1 and 2). With this particular mAb, it was necessary to run the column at low pH (pH 5.0) and high salt (0.4 M NaCl) conditions. At these conditions the HCP removal is limited. Typically, when the mAb allows running at higher pH and lower salt conditions, Capto™ adhere also removes HCPs.

Conclusions

The performance of ReadyToProcess™ columns is comparable with established column formats as has been demonstrated in a three-step mAb purification process run in parallel at two different scales; small-scale XK columns and large-scale, prepacked ReadyToProcess™ columns. The ReadyToProcess™ columns behave similarly to the XK columns in all aspects studied, demonstrating that the purification process is directly scalable between XK and ReadyToProcess™. Multiple cycles (five) have been performed on ReadyToProcess™ 2.5 L column packed with MabSelect SuRe™ without any detectable changes in column performance.

References

1. Steindl *et al.* A simple method to quantify staphylococcal protein A in the presence of human or animal IgG in various samples. *J. Immunol. Methods* 235, 61-69 (2000).

Column:	Capto™ Q, 40 mL in XK16/40 (A) and ReadyToProcess™ 2.5 L column packed with Capto™ Q (B), bed height 20 cm
Equilibration buffer:	0.01 M sodium phosphate, 0.01 M sodium citrate pH 7.6
Sample:	Pooled, pH-adjusted eluates from MabSelect SuRe™
Sample load:	119 mg mAb/mL resin (A, 8.4 CV), 116 mg mAb/mL resin (B, 10.1 CV)
Wash:	Equilibration buffer (5 CV)
Elution:	0.1 M acetic acid
pH adjustment:	Equilibration buffer (1 CV)
CIP:	0.5 M NaOH (3 CV)
Re-equilibration:	Equilibration buffer (3 CV)
Liquid velocity:	500 cm/h
System:	ÄKTAexplorer™ 100 (A), ÄKTAprocess™ (B)

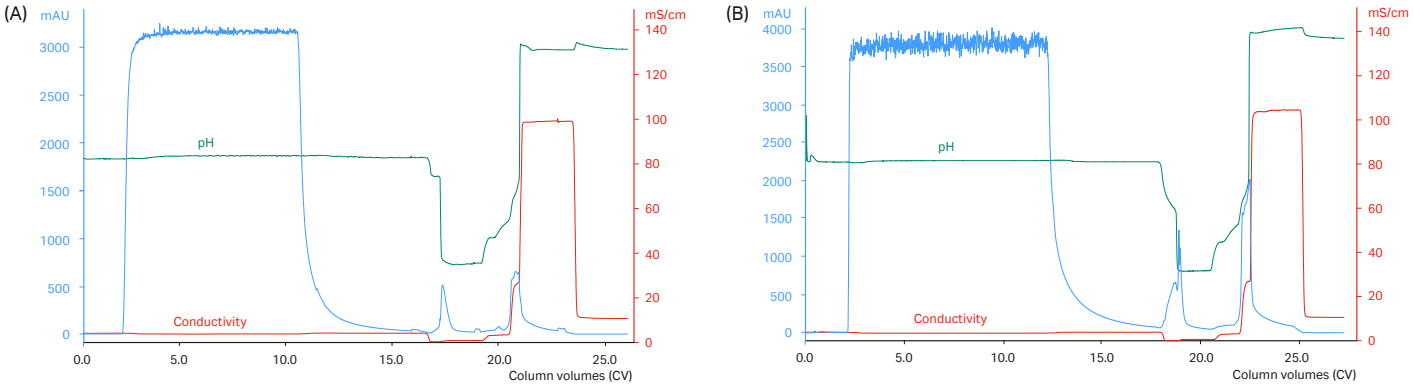

Fig 2. Comparison of the interresinste purification step using **(A)** an XK 16/40 column packed with Capto™ Q and **(B)** a prepacked ReadyToProcess™ 2.5 L column packed with Capto™ Q.

Column:	Capto™ adhere, 40 mL in XK16/40 (A) and ReadyToProcess™ 2.5 L column packed with Capto™ adhere (B), bed height 20 cm
Equilibration buffer:	0.01 M sodium phosphate, 0.02 M sodium citrate, 0.4 M sodium chloride pH 5.0
Sample:	pH- and conductivity-adjusted flowthrough fraction from Capto™ Q
Sample load:	112 mg mAb/mL resin (A, 11 CV), 120 mg mAb/mL resin (B, 13.2 CV)
Wash:	Equilibration buffer (10 CV)
Elution:	0.1 M acetic acid
pH adjustment:	Equilibration buffer (1 CV)
CIP:	0.5 M NaOH (3 CV)
Re-equilibration:	Equilibration buffer (3 CV)
Liquid velocity:	500 cm/h
System:	ÄKTAexplorer™ 100 (A), ÄKTAprocess™ (B)

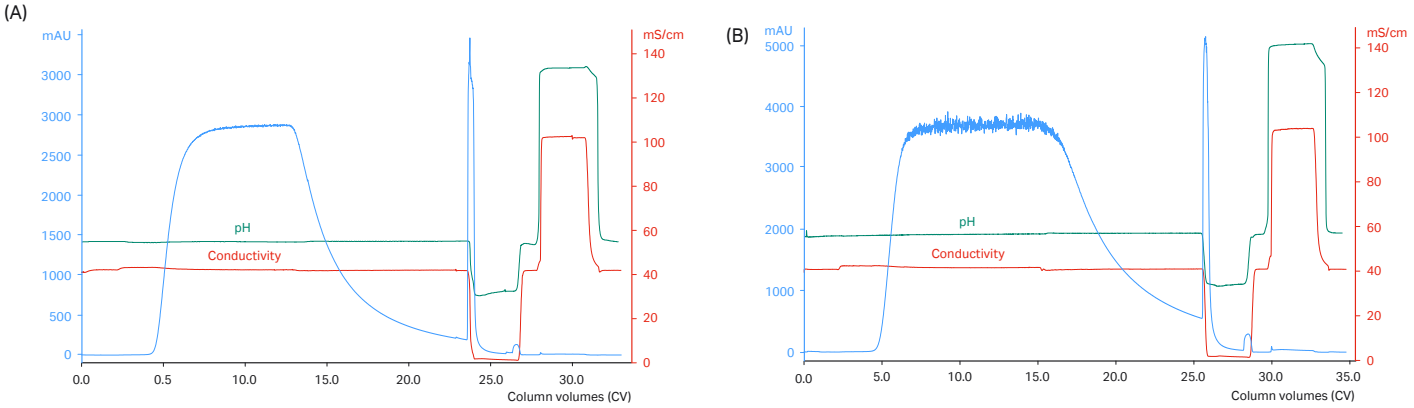

Fig 3. Comparison of the polishing step using **(A)** an XK 16/40 column packed with Capto™ adhere and **(B)** a prepacked ReadyToProcess™ 2.5 L column packed with Capto™ adhere.

Ordering information

Product	Quantity	Code No.
XK 16/40 column	1	18 8774 01
MabSelect SuRe™ resin	25 mL	17 5438 02
Capto™ Q resin	25 mL	17 5316 10
Capto™ adhere resin	25 mL	17 5444 01
ReadyToProcess™ Capto™ Q 2.5 L (126/200)	2.5 L	28 9017 23
ReadyToProcess™ Capto™ Q 10 L (251/200)	10 L	28 9017 24
ReadyToProcess™ Capto™ Q 20 L (359/200)	20 L	28 9017 25
ReadyToProcess™ Capto™ adhere 2.5 L (126/200)	2.5 L	28 9017 14
ReadyToProcess™ Capto™ Q 10 L (251/200)	10 L	28 9017 15
ReadyToProcess™ Capto™ adhere 20 L (359/200)	20 L	28 9017 16
ReadyToProcess™ MabSelect SuRe™ 2.5 L (126/200)	2.5 L	28 9017 17
ReadyToProcess™ MabSelect SuRe™ 10 L (251/200)	10 L	28 9017 18
ReadyToProcess™ MabSelect SuRe™ 20 L (359/200)	20 L	28 9017 19

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