TOSOH

Application Note

Reproducible Protein A Capture from Development to Large Scale with Pre-packed Columns

Protein A chromatography is the standard capture step for monoclonal antibody (mAb) purification due to its high selectivity and robustness. When developing mAb purification processes, users need to scale from early development to manufacturing scale without changing the underlying chromatographic behavior or compromising product quality. This requires chromatography columns that deliver **reliable and reproducible performance across different column sizes**.

The SkillPak™ platform is designed to support straightforward scale-up by providing pre-packed columns with defined and reproducible packing quality. SkillPak 5 and SkillPak 50 are commonly used for early development and pilot-scale work, while SkillPak 1k MAX extends this concept to large scale purification. Using the same chromatographic base across formats facilitates predictable process transfer and reduces development risk.

In this application note, the scalability and robustness of the SkillPak platform are demonstrated using a trastuzumab feedstock. Purification performance on SkillPak 1k MAX Super A is evaluated and compared with results obtained using SkillPak 5 Super A and SkillPak 50 Super A. The results show that the SkillPak platform enables **easy scale-up while maintaining reliable and reproducible performance**.

Characterizing Purification Performance

Experimental Conditions

Table 1. Purification Method

Equilibration buffer	20 mmol/L sodium phosphate + 150 mmol/L NaCl, pH 7.4
Wash buffer 2	25 mmol/L sodium citrate + 150 mmol/L NaCl, pH 5.2
Elution buffer	25 mmol/L sodium citrate, pH 3.2
CIP	250 mmol/L NaOH
Load material	Trastuzumab feedstock, approximately 9.43 g/L, filtered (0.45 µm)
Load	80% DBC of TOYOPEARL® Super A resin
Linear velocity	240 cm/h
Residence time	5 min

Results and Discussion

Purification performance of SkillPak 1k MAX Super A was evaluated over two purification runs using a trastuzumab feedstock with a concentration of 9.43 g/L and a load of 80% DBC of the TOYOPEARL Super A resin. Across both runs, the column consistently delivered high product recovery, demonstrating reproducible capture efficiency at large scale. Recovery values above 98% were achieved in both purification runs, confirming reliable performance of the Protein A capture step *(Table 2)*.

Table 2. Purification results – SkillPak 1k MAX Super A

Parameter	Load	Run 1	Run 2	Average Run 1 and Run 2
Recovery (%)		98.24	99.18	98.71
Monomer content (%)		94.37	95.12	94.74
HMW content (%)		4.26	3.73	3.99
LMW content (%)		1.37	1.16	1.26
Residual HCP (ppm)	46.47	1.45	1.43	1.44

Table 2 shows that product quality attributes were consistently improved compared to the load material. Monomer content increased substantially after purification, while low molecular weight (LMW) species were effectively reduced to approx. 1.3 %. The comparable reduction of impurities observed in both runs indicates stable separation performance and reproducible interaction between the antibody and the Protein A ligand.

Residual host cell protein (HCP) levels after purification were low and comparable between the two purification runs, demonstrating reliable impurity clearance. Overlay of chromatograms *(Figure 1)* showed consistent elution profiles and peak shapes between runs, confirming run-to-run reproducibility of the chromatographic behavior.

An increase in system pressure was observed during the loading phase of the second purification run *(Figure 2)*. This was attributed to feedstock handling rather than changes in column performance, as the feedstock for the first run was filtered immediately before purification, while the feedstock of run 2 was stored after filtration for 4.5 hours before purification. Column efficiency and purification outcomes remained stable, further demonstrating the robustness of SkillPak 1k MAX Super A under large scale operating conditions.

Figure 1. Purification Run 1 & 2 Overlay chromatogram

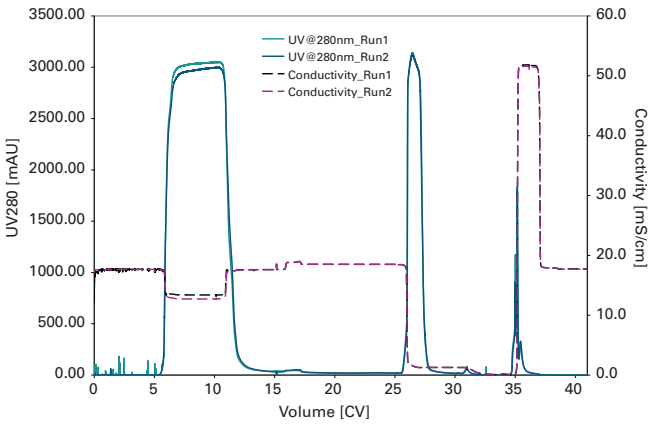
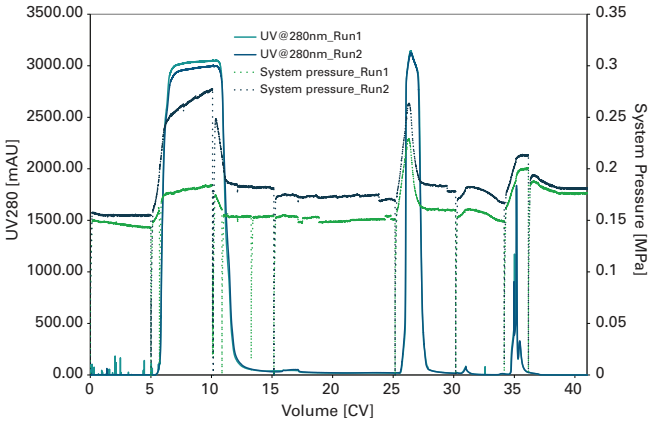


Figure 2. Purification Run 1 & 2 chromatogram with pressure overlay



Column Performance testing

Experimental Conditions

Columns: SkillPak 1k MAX Super A
(8cm ID × 20 cm bed height)
Mobile phase: 1 mol/L NaCl
Linear velocity: 100 cm/h
Injection: 3 mol/L NaCl, 10 mL (loop injection)

Results and Discussion

Column performance testing was carried out prior to the purification runs to verify packing quality and establish baseline column efficiency as well as after the purification runs and the cleaning-in-place. Performance was assessed by measuring column asymmetry and reduced plate height using a standard tracer injection method. Results are shown in *Table 3*.

Table 3. Column performance before & after purification

Parameter	Specification	Result before purification	Result after purifications
Column asymmetry	0.80 – 1.40	1.27	1.30
Reduced plate height	≤ 8.0	2.81	2.75

Column asymmetry and reduced plate height values remained comparable to those measured before purification, indicating that neither purification nor cleaning adversely affected column efficiency or bed integrity.

The consistency of performance parameters before and after purification demonstrates the robustness and mechanical stability of the packed bed and confirms that SkillPak 1k MAX Super A maintains a reliable and reproducible chromatographic performance, supporting repeated use.

Easy Scale Up Within the SkillPak Platform

Experimental Conditions

Columns: SkillPak 5 Super A
(5 mL column, 8.0 mm ID × 10 cm)
SkillPak 50 Super A
(50 mL column, 2.5 cm ID × 10 cm)

Same lot of TOYOPEARL Super A resin was used across all columns (SkillPak 5, SkillPak 50, SkillPak 1k Max)

Table 4. Purification Method

Equilibration buffer	20 mmol/L sodium phosphate + 150 mmol/L NaCl, pH 7.4
Wash buffer 2	25 mmol/L sodium citrate + 150 mmol/L NaCl, pH 5.2
Elution buffer	25 mmol/L sodium citrate, pH 3.2
CIP	250 mmol/L NaOH
Load material	Trastuzumab feedstock, approximately 9.43 g/L, filtered (0.45 µm)
Load	80% DBC of TOYOPEARL Super A resin
Linear velocity*	240 cm/h
Residence time	5 min

*Flow rates were adjusted to 120 cm/h for SkillPak 5 and SkillPak 50 to maintain comparable residence times across the different column sizes.

Results and Discussion

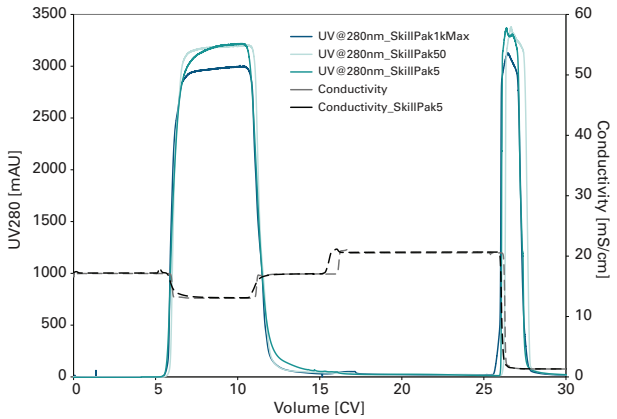
To evaluate ease of scale up within the SkillPak platform, purification performance obtained with SkillPak 1k MAX Super A was compared with results generated using SkillPak 5 and SkillPak 50 columns. Therefore, the same method and the same feedstock used for the purification with the SkillPak 1k Max column was used for SkillPak 5 and SkillPak 50, with an adjusted flow rate of 120 cm/h, to keep residence times constant. Across all column formats, comparable purification behavior was observed, including similar monomer content, effective LMW content reduction, and consistent recovery. Results are summarized in *Table 5*.

The SkillPak 1k MAX Super A column delivered higher eluate concentration while maintaining product quality attributes comparable to smaller SkillPak formats. These results demonstrate that the Protein A capture process can be transferred from SkillPak 5 and SkillPak 50 to SkillPak 1k MAX Super A without fundamental process changes. *Figure 3* shows an overlay the different runs of SkillPak 5, SkillPak 50 and SkillPak 1k MAX for a better comparison.

Table 5. Comparison of purification performance across SkillPak formats

Parameter	SkillPak 5	SkillPak 50	SkillPak 1k MAX
Eluate concentration (mg/mL)	15.15	15.57	18.42
Recovery (%)	96.78	97.58	98.71
Monomer content (%)	94.11	93.10	94.74
HMW content (%)	4.63	5.59	3.99
LMW content (%)	1.32	1.32	1.26

Figure 3. Overlay of purification chromatograms across SkillPak 5, 50, and 1k MAX



These results confirm that the SkillPak platform supports **straight forward scale-up**, allowing users to move from SkillPak 5 to SkillPak 50 and SkillPak 1k MAX Super A without compromising chromatographic performance.

Conclusions

The SkillPak platform enables **easy and reliable scale-up of Protein A capture** for monoclonal antibody purification. SkillPak 5 Super A and SkillPak 50 Super A support early development and pilot-scale operation, while SkillPak 1K MAX Super A extends the same reproducible chromatographic performance to large scale.

Consistent column efficiency before and after purification, reproducible recovery, and stable product quality confirm that SkillPak 1k MAX Super A provides a robust solution for large scale Protein A capture. Users benefit from **predictable scale-up, reduced operational risk, and reliable purification performance.**

Featured Products

Part #	Description
0045400	SkillPak 5 Super A, 5 mL column, 8.0 × 100 mm
0045401	SkillPak 50 Super A, 50 mL column, 2.5 × 10 cm
0045601	SkillPak 1k MAX Super A, 1005 mL column, 8.0 × 20 cm