JSR Life Sciences

Platform purification of six biosimilar molecules using Amsphere A3 – protein A resin

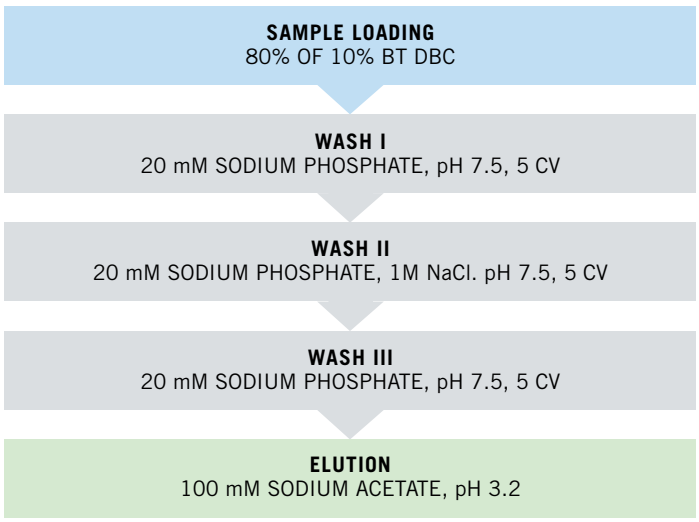
Currently more than 70 biosimilar mAbs (monoclonal antibodies) are under development and multiple originator mAbs are going off-patent in the next 3-4 years. Protein A resin remains the most important workhorse for the purification of monoclonal antibodies. Protein A resin has a high impact on both development and manufacturing cost, in particular during early stage clinical phases. This application note summarizes the key performance parameters for our high capacity protein A resin, Amsphere A3, for 6 biosimilar molecules of which five are mAbs and one is a Fc-fusion protein.

Materials and Methods

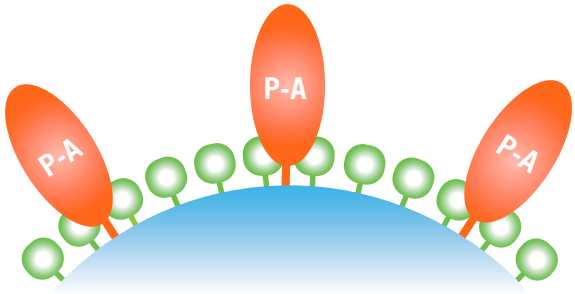
TABLE 1: MONOCLONAL ANTIBODIES AND FUSION PROTEIN—ALL BIOSIMILARS DERIVED FROM CHO CELL LINE CLARIFIED BY CENTRIFUGATION AND 0.22 MICROMETER FILTRATION

LABEL	MOLECULE
mAb1	Trastuzumab
mAb2	Adalimumab
mAb3	Bevacizumab
mAb4	Palivizumab
mAb5	Rituximab
Fc-fusion protein	Etanercept

FIGURE 1: TEST CONDITIONS OF CHROMATOGRAPHY PROCESS



Amsphere™ A3



Amsphere A3 is a new protein A resin designed with a surface modified base bead and alkali-resistant optimized ligand.

Protein A ligand

- High DBC via controlled conformation and orientation
- High alkaline stability from protein engineering

Surface modification

- Low HCP levels by surface hydrophilization

Base bead formulation

- High DBC at high flow rate
- Excellent pressure and flow properties via rigid crosslinking

TABLE 2: EXPERIMENTAL METHODS AND MATERIALS

ITEM	DETAILS
Column	0.5 x 5 cm (1 mL); Except DBC (mAb1) 0.5 x 20 cm (4 mL)
Residence time	5 min; Except DBC (mAb1): 4 min
Detection	UV at 280 nm
Sample	mAb in CHO clarified cell culture fluid

Results

FIGURE 2: PLATFORM PURIFICATION OF FIVE BIOSIMILAR ANTIBODIES USING AMSPHERE A3

Figure 2 depicts the dynamic binding capacity (DBC) for 4 mAbs and 1 Fc-fusion protein. The DBC for mAb5 (Rituximab) was not determined.

The DBC was consistently between 43 g/L and 57 g/L at a residence time of 5 min (except for Fc-fusion protein).

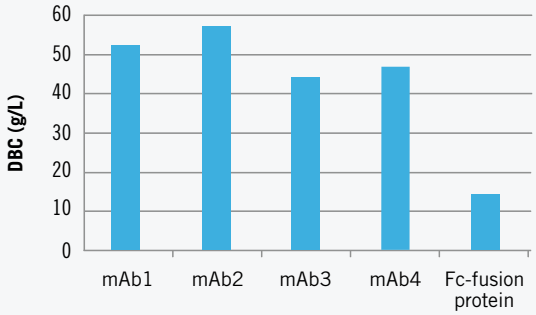


TABLE 3: HCP REMOVAL

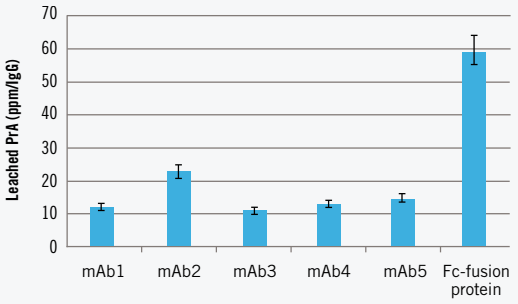
Table 3 shows the HCP removal which was consistently more than 2 LRV (Log Reduction Value) for all mAbs. The Fc-fusion protein showed approximately 1.8 LRV.

MOLECULE	HCCF (ppm)	ELUATE (ppm)	LRV
mAb1	350,000	3,100	2.05
mAb2	220,000	1,700	2.10
mAb3	315,000	3,850	1.91
mAb4	1,170,000	1,650	2.85
mAb5	560,000	2,950	2.27
Fc-fusion protein	628,000	10,200	1.78

**Detection by HCP ELISA (Cygnus F550)*

FIGURE 3: LEACHED PROTEIN A

In Figure 3, leached protein A was between 10 and 20 ppm for all mAb feedstocks. The Fc-fusion protein showed higher leaching at approximately 58 ppm. In all cases leached protein A is expected to drop after a subsequent polishing step.



**Detection by Protein A ELISA (Cygnus F740)*

Discussion and Conclusions

Amsphere A3 consistently delivers high binding capacities and excellent impurity clearance for multiple biosimilar molecules as highlighted in this application note. Amsphere A3 excels with comparably high DBC demonstrating its potential to improve the process economics for biosimilar development and manufacturing. In addition, the data underlines the suitability of Amsphere A3 as a platform, since the same protocol was used for all investigated mAbs, consistently yielding excellent performance results with standard wash and elution buffers.

For more information visit www.jsrlifesciences.com

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