Advanced affinity chromatography tools for bispecific antibody purification

Jett Appel, David Humphries
Thermo Fisher Scientific, Bedford, MA 01730 USA

Abstract

Bispecific antibodies (bsAbs) are engineered to simultaneously bind two different antigens or epitopes, offering unique advantages in cancer treatment or immunotherapy. Due to their complex structures, the production of these antibodies is often prone to high levels of product related impurities that are challenging to separate, including mispaired species, aggregates, half antibodies, free light chains (LC), and LC dimers.

Affinity chromatography resins targeting various antibody subdomains have been implemented to address these challenges.

Two case studies from the literature are presented here, where yield and purity of the target molecule are improved over standard affinity chromatography techniques.

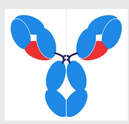
Case study 1

Mispaired species removal in bsAb production by LC binding avidity

This case study was published by Rezvani et al in the Journal of Chromatography A (ref 1).

Introduction

The production of therapeutic asymmetric bsAb formats can result in mispaired species, examples shown below:

Target construct	Example mispaired impurity species		
BsAb	λ-λ Homodimer	κ-κ Homodimer	κ-κ Heterodimer
			

Several light chain affinity resins were evaluated for the effective separation of these impurities for three different therapeutics asymmetric bsAbs.

Materials and methods

Dynamic binding capacity: 10% breakthrough measured at 4 min residence time with purified bsAb

pH gradient elution: Loading at 10 mg bsAb/mL-resin, 20 column volume (CV) linear gradient elution with 50 mM sodium citrate/citric acid, pH 6.0 – 2.5 at 4 min residence time, with or without modifiers

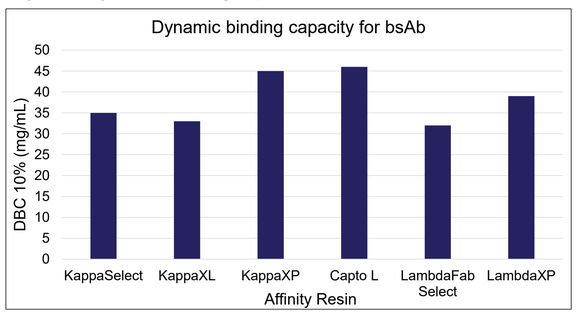
Samples analyzed by HPLC (ProA or Lambda LC, SEC, HIC), and non-reduced capillary gel electrophoresis (NR-CGE)

Results

Dynamic binding capacity data

Thermo Scientific™ CaptureSelect™ KappaXP, Capto L, and Thermo Scientific™ CaptureSelect™ LambdaXP resins provided the highest capacities for a purified bsAb at 4-minute residence times.

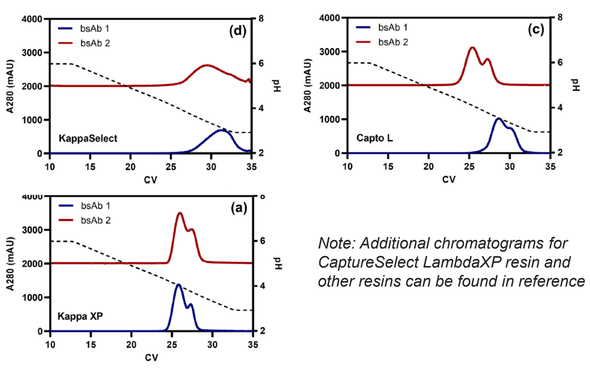
Figure 1. Dynamic binding capacities of resins evaluated



pH gradient elution on light chain resins

pH gradient experiments showed that overall CaptureSelect KappaXP and CaptureSelect LambdaXP resins provided the greatest resolution of target molecule and product variants.

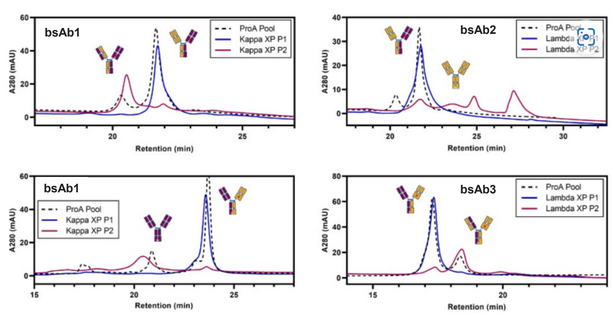
Figure 2. Example chromatograms demonstrate best resolution among the evaluated resins for both bsAb1 and 2 with CaptureSelect KappaXP resin



Note: Additional chromatograms for CaptureSelect LambdaXP resin and other resins can be found in reference 1

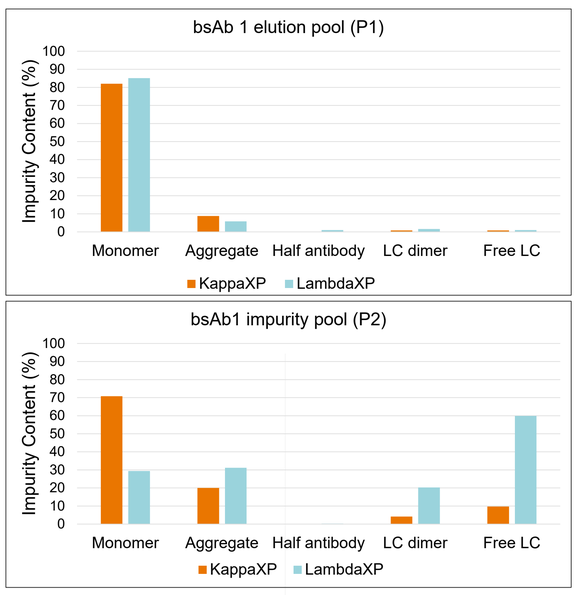
HIC-HPLC results demonstrate substantial removal of mispaired species in the purified pool (blue trace) that are co-purified by Protein A (dashed trace).

Figure 3. HIC-HPLC absorbance traces of collected fractions from LC affinity pH gradient elution experiments of bsAb feeds



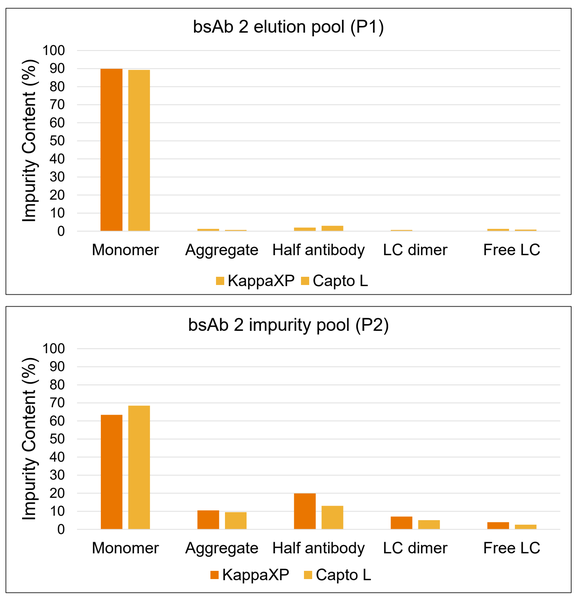
Further investigation by HP-SEC and NR-CGE analysis of main peak (P1) and peak 2 (P2) confirm the main pool is comprised of the target heterodimer with lower levels of residual aggregates, while the late eluting peak is enriched in aggregates and fragments such as LC dimers and free LCs.

Figure 4. Composition of elution pool and impurity pool for bsAb1



Similar trends are observed for bsAbs 2 and 3. BsAb2 data demonstrated separation of half antibody, LC dimer, and free LC impurities through kappa LC affinity purification.

Figure 5. Composition of elution pool and impurity pool for bsAb2



Impact of elution modifiers:

For bsAb2 with CaptureSelect KappaXP resin, modifiers improved resolution of the heterodimer from the homodimer (figure 6). This required higher salt concentrations which may impact yield and stability (figure 7). More optimization is required if modifiers are implemented.

Figure 6. pH gradient elution peak pH values on KappaXP resin using modifiers. The use of $MgCl_2$ provided the best improvement in resolution with the κ-homodimer eluted only in the pH 2.8 strip*

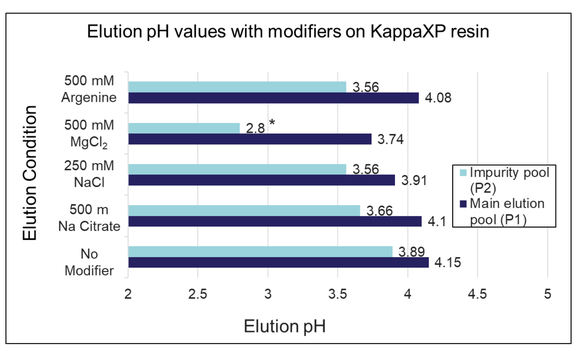
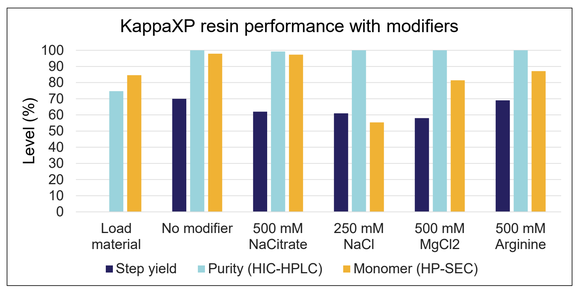


Figure 7. Effect of elution modifiers on purification of bsAb2 with CaptureSelect KappaXP resin



Resolution with CaptureSelect LambdaXP resin was improved with low levels of modifiers for bsAb1 and bsAb3. Chromatogram and performance data for bsAb1 with 20 mM NaCl is shown. Load material was lambda affinity purified material. All modifiers removed mispaired species (98% pure) and aggregates (~95-97% monomer).

Figure 8. Elution chromatogram of bsAb1 purified on CaptureSelect LambdaXP resin with pH step elution and with and without modifiers

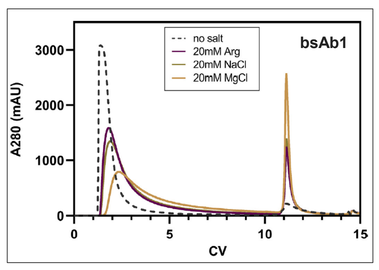
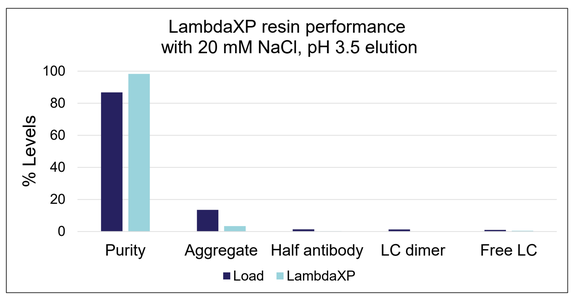


Figure 9. Effect of addition of 20mM NaCl in elution buffer, pH 3.5 on CaptureSelect LambdaXP resin purification of BsAb1



Conclusions

- CaptureSelect KappaXP and CaptureSelect LambdaXP resins provided the greatest resolution of product from mispaired variants.
- CaptureSelect resins additionally demonstrated capabilities to remove aggregates, fragments, half antibodies, free LCs, and LC dimers with substantial monomer recoveries.
- Multivalently bound species and elution modifiers may be leveraged to enhance separation of the target molecule from mispaired species.

Case study 2

Alternative affinity capture for the purification of CH1-containing bsAbs

This case study was published by Chamow et al in the BioProcess International (ref 2).

Introduction

The purification of bsAbs by standard protein A (ProA) affinity chromatography is challenged by the coelution of product-related impurities that contain Fc regions with the target molecule. In addition, low pH conditions required to elute from ProA can cause high levels of aggregation for some bsAb formats.

In the studies by Chamow et al, the purification of BsAb CD3-TAA via ProA affinity chromatography was ineffective for these reasons.

BsAb CD3-TAA is a fully human IgG4 containing two heavy chains and one kappa light chain, utilizing knobs into holes technology. One arm contains two identical VH domains that bind a tumor-associated antigen (TAA), lacking a CH1 domain; the other CH1-containing arm targets CD3. The active form and mispaired species are shown below:

Active form	Product-related impurity species			
BsAb	TAA homodimer	CD3 homodimer	Half antibody	Free light chain
				

Thermo Scientific™ CaptureSelect™ CH1-XL affinity resin was evaluated as an alternative because of its specificity to the CH1 region and mild elution conditions compared to ProA. This allows for non-binding of the TAA homodimer, a prominently present impurity, as well as reduced aggregation levels.

Materials and methods

ProA purification: MabSelect Sure LX – 1 mL, 50 mL HCCF load, EQ 50 mM Tris, pH 7.0, EQ wash, elute 25 mM citric acid, pH 3.6

CH1-XL resin purification: 9 mL, 50 mL HCCF load, EQ wash, 50 mM Tris, 0.5 M NaCl, pH 7.0 wash, elute 50 mM acetic acid, 10% glycerol, and 10% sucrose, pH 4.0

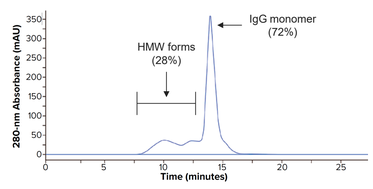
Samples analyzed by SDS-PAGE, UHPLC, and IEF

Results

Protein A resin purification

Purification of bsAb CD3-TAA by ProA was efficient (elution pH < 3.6) but resulted in high levels of aggregation (28%) and coelution of homodimer variants. Additives like polyols helped reduce aggregation, but levels were still substantial. Higher elution pH values resulted in inefficient recovery.

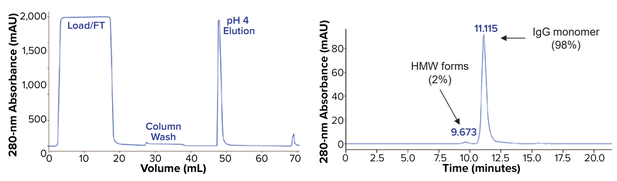
Figure 10. HPLC analysis of ProA eluate (pH 3.6)



CaptureSelect CH1-XL resin purification

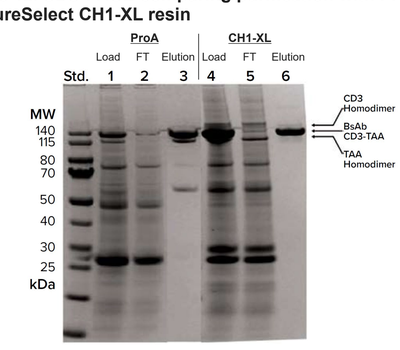
Purification of bsAb CD3-TAA with CaptureSelect CH1-XL resin was effective at separating product from variants and resulted in significantly lower levels of aggregation at milder elution conditions and with the addition of modifiers.

Figure 11. Purification by CaptureSelect CH1-XL resin (elution: 50 mM acetic acid, 10% glycerol, 10% sucrose, pH 4) and UHPLC analysis



Comparison of purification by ProA and CaptureSelect CH1-XL resin by SDS-PAGE confirms the above results, showing the elution pool from ProA (lane 3) containing both homodimers and aggregate impurities, where purification by CaptureSelect CH1-XL resin clearly shows the TAA-homodimer in the flow-through fraction (lane 5).

Figure 12. SDS-PAGE comparing purification with ProA vs CaptureSelect CH1-XL resin



Conclusions

- CaptureSelect CH1-XL resin provided milder elution conditions (pH 4) with higher monomer recovery and purity compared to ProA.
- CaptureSelect CH1-XL resin provided separation from homodimer impurities due to affinity targeting the CH1 domain.
- CaptureSelect CH1-XL resin was implemented in clinical production of bsAb drug substance.

References

1. Rezvani, W. et al (2022). Leveraging light chain binding avidity for control of mispaired byproducts during production of asymmetric bispecific antibodies, *Journal of Chromatography A,* 1683, 463533. https://doi.org/10.1016/j.chroma.2022.463533.
2. Chamow, S. et al (2020). Capture of CH1-Containing Bispecific Antibodies, *BioProcess International,* https://www.bioprocessintl.com/chromatography/capture-of-ch1-containing-bispecific-antibodies-evaluating-an-alternative-to-protein-a

Trademarks/licensing

Science at a scan
Scan the QR code on the right with your mobile device to download this and many more scientific posters.

Learn more at thermofisher.com/antibody-therapeutics