Improved Process Economics of HUMIRA Biosimilar Purification with Ion Exchange and Mixed-Mode Resins

Randy Drevland, Chelsea Pratt, Payal Khandelwal, Laura Moriarty, and Elizabeth Jordan Dreskin
Bio-Rad Laboratories, Inc., 6000 Alfred Noble Drive, Hercules, CA 94547

Abstract

The global biosimilars market is exploding and is expected to reach US$10.9 billion by 2021. Biosimilars are significantly cheaper than biologics, owing to their shorter development time and fewer involved costs. However, further reduction in their development costs is required for broader affordability. Biosimilar purification costs constitute more than half of the total development costs. A significant number of these costs are due to the expense of Protein A–based media. Here, we propose an alternate but equally effective approach to biosimilar purification, by substituting a less expensive ion exchange resin for the affinity capture step. With an adalimumab (HUMIRA) biosimilar, we show that using a Nuvia S Cation Exchange Resin can not only help minimize purification costs, but also overcome the limitations of Protein A capture, including low binding capacity and Protein A leaching. In addition, using the IEX-based workflow in conjunction with a mixed-mode resin polish step can help achieve greater than 99% purity of the biosimilar, which is comparable to a Protein A–based purification. Thus, an IEX-based process works as a great alternative to traditional biosimilar purifications and leads to improved process economics, which is an underlying requirement of a biosimilar.

Introduction

The WHO defines biosimilars as “a biotherapeutic product which is similar in terms of quality, safety and efficacy to an already licensed reference biotherapeutic product” (World Health Organization 2009). The goal of developing a biosimilar is to have a cheaper but equally efficacious alternative to the already licensed reference biotherapeutic product, or originator biologic, since biologics development involves, on average, over a decade of time investment and approximately US$2.6 billion (DiMasi et al. 2016). Biosimilars show promise as an alternative to originator biologics with cost-saving predictions reaching US$250 billion in the next ten years (Jacoby et al. 2015). These savings, at least in part, are due to the fact that the development of a biosimilar takes 7–8 years, at a cost between US$100 and 250 million (Blackstone and Joseph 2013).

It is noteworthy that over 50% of the total costs required for biosimilar development come from the preclinical phase (Gutierrez 2015). A significant part of the preclinical phase comprises purifying an active biomolecule and downstream bioanalytical characterization steps. Protein A purification platforms are often used for these purification steps. However, there are multiple limitations to using Protein A affinity resins. They are expensive and have low dynamic binding capacities (DBC), which results in a limited amount of purified sample per unit of resin. Some of the newer Protein A resins with higher DBCs lead to increased protein aggregation, which also leads to decreased productivity. Leaching of Protein A from resins often further complicates the purification process, requiring additional steps to remove leached material and additional QC assays to test that the leached material has been removed.

In complete contrast, ion exchange resins are cheap, have high DBCs, can be used for more cycles, and can be cleaned and stored with more aggressive solutions. They therefore offer a potential for cost saving, provided the end product can be shown to be similar to the originator.

In this study, we performed parallel capture of a HUMIRA biosimilar with a Protein A affinity resin and a next-generation ultra-high capacity cation exchange (CEX) resin, Nuvia S. The purification workflow (Figure 1) included the anion exchanger Nuvia Q for the intermediate polish step and the mixed-mode media Nuvia cPrime for the final polish purification. Our results demonstrate that the IEX-based workflow resulted in greater than 99% purity of the biosimilar fraction, which is equal to that expected from the Protein A affinity resin workflow. In addition, the biosimilar purified from the IEX workflow retained its structure and function. We also show the comparative cost analysis of both workflows to demonstrate the improved process economics of using the IEX-based workflow.

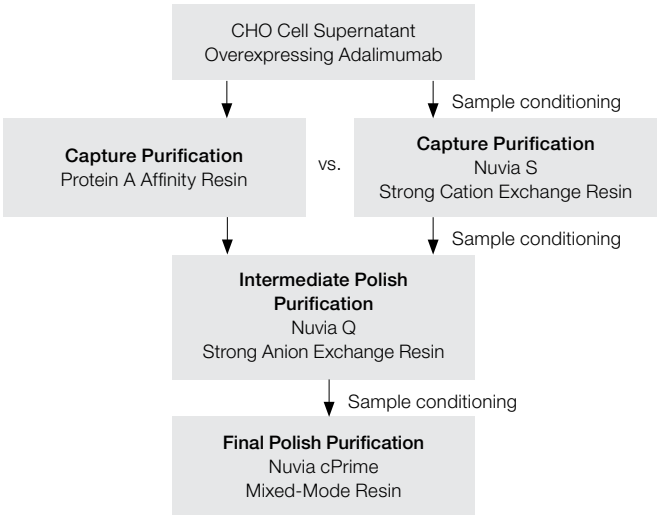


Fig. 1. Adalimumab biosimilar purification workflow.

Materials and Methods

General

An adalimumab biosimilar produced in Chinese hamster ovary (CHO) cells was purchased (Syd Labs, catalog #C003P). Protein fractions were analyzed by SDS-PAGE using Criterion Tris-HCl 8–16% Linear Gradient Gels (Bio-Rad Laboratories, #3450037) stained with Bio-Safe Coomassie Stain (Bio-Rad, #1610786). HCP clearance was determined using the CHO HCP ELISA Kit (Cygnus Technologies, #F550) and host cell DNA (hcDNA) was quantified using the ddPCR CHO Residual DNA Quantification Kit (Bio-Rad, #17000031). Preliminary chromatography experiments to compare both the workflows were conducted in 96-well plates (Pall Corporation, #8119). Larger scale purifications were conducted using 1 ml columns filled with the appropriate resins.

Capture by Nuvia S CEX Resin

Chromatographic purification was conducted using the NGC Chromatography System (Bio-Rad, #7880001). A 1 ml Foresight Nuvia S Column (Bio-Rad, #732-4720) was equilibrated with 10 column volumes (CV) of 25 mM sodium acetate (pH 4.5), 5 mM NaCl (buffer A). The CHO cell supernatant containing the biosimilar was adjusted to pH 4.5 with 1 M hydrochloric acid (HCl) and applied to the pre-equilibrated Nuvia S Column at a flow rate of ~120 cm/hr. The column was washed with 5 CV of buffer A followed by 5 CV of 25 mM sodium acetate (pH 4.75), 75 mM NaCl. The bound biosimilar was eluted with 10 CV of 25 mM sodium acetate (pH 5.0), 250 mM NaCl (buffer B).

Capture by Protein A Affinity Resin

MabSelect SuRe Protein A Resin (GE Healthcare, #17-5438-01) was packed in a 1 ml column according to the manufacturer's instructions and the biosimilar was loaded onto it. The bound biosimilar was eluted with 10 CV of 100 mM sodium acetate (pH 3.5), 100 mM NaCl.

Intermediate Polishing by Nuvia Q AEX Resin

The eluate from the capture step was concentrated and buffer exchanged into 25 mM HEPES (pH 8.25), 5 mM NaCl (buffer C) using an Amicon Ultra-4 Centrifugal Filter Unit (50 kD MW cutoff) (EMD Millipore, #UFC805024). This sample was applied on a 1 ml Foresight Nuvia Q Column (Bio-Rad, #732-4721) equilibrated with 10 CV of buffer C. The flow-through fractions were collected for further purification.

Final Polishing by Nuvia cPrime Mixed-Mode Media

The pooled flow-through fractions from the previous step were concentrated, buffer exchanged with buffer B, and applied to a Foresight Nuvia cPrime Column (Bio-Rad, #732-4722) pre-equilibrated with 10 CV of the same buffer. The biosimilar was eluted with 5 CV of 25 mM Tris (pH 8.0), 250 mM NaCl. The column was then stripped with 10 CV of 25 mM Tris (pH 8.0), 800 mM NaCl to remove all bound impurities.

Adalimumab Activity Assays

Pharmacokinetic ELISA Antigen Capture Format

A 96-well black flat-bottom plate was coated with 100 µl of 5 µg/ml TNFα, incubated overnight at 4°C, and then washed five times with phosphate buffered saline with Tween-20 (PBST). Each well was blocked with 300 µl 5% BSA in PBST for one hour at room temperature. The plate was then washed five times with PBST, and 100 µl of each sample was added in triplicate. The plate was incubated for one hour at room temperature and then washed five times with PBST. Anti-adalimumab conjugated with HRP (Bio-Rad, #HCA232P) was diluted to 2 µg/ml in HISPEC buffer (Bio-Rad, #BUF049) and 100 µl of the detection antibody was added to each well. The plate was incubated for one hour at room temperature and then washed ten times with PBST. QuantaBlu Fluorogenic Peroxidase Substrate (100 µl) (Thermo Fisher Scientific, #15169) was added to each well and the fluorescence was measured after 30 minutes. The concentration of purified adalimumab was calculated from a standard curve of adalimumab prepared in 10% human serum in PBST.

Pharmacokinetic Bridging ELISA

An anti-adalimumab capture antibody (Bio-Rad, #HCA202; AbD18654) was diluted to 1 µg/ml in PBS, and 100 µl of the antibody was added to each well of a 96-well black flat-bottom plate. The plate was incubated overnight at 4°C and then washed five times with PBST. Each well was then blocked with 300 µl 5% BSA in PBST. The plate was incubated for one hour at room temperature and then washed five times with PBST. Each sample (100 µl) was added in triplicate and incubated for one hour at room temperature. The plate was then washed five times with PBST. The HRP-conjugated detection antibody HCA204P (Bio-Rad, #AbD18655_hIgG1) was diluted to 2 µg/ml in HISPEC buffer and 100 µl of the antibody was added to each well. The plate was incubated for one hour at room temperature and then washed ten times with PBST. QuantaBlu Substrate (100 µl) was added to each well and the fluorescence measured after 30 minutes. The concentration of purified adalimumab was calculated from a standard curve of adalimumab prepared in 10% human serum in PBST.

Biological Assay to Assess Adalimumab Blocking TNFα

Murine L929 cells were plated in growth media (MEM, 2 mM glutamine, 10% FBS) and kept at 37°C in 5% CO_2. Anti-TNFα antibody (either control adalimumab or adalimumab prepared from lysate) was prepared in serum-free media and added to cells at a final concentration of 300 ng/ml. Following a two hour incubation with the antibodies, TNFα was added at a final concentration of 5 ng/ml, and the cells were incubated for a further 36 hours at 37°C in 5% CO_2.

VivaFix Cell Viability Staining

VivaFix Cell Viability Dyes fluoresce more brightly in dead cells than live ones. In live cells, they bind only to cell surface primary amines. In dead cells, the compromised membrane allows additional binding to intracellular primary amines. Staining with VivaFix Dyes was performed according to the manufacturer's instructions. Briefly, TNFα-treated cells were washed with PBS, trypsinized, and neutralized, and cell suspensions were added to microcentrifuge tubes. The cells were counted and centrifuged at 300 RCF. The supernatant was removed and the cells were resuspended in PBS. VivaFix 353/442 (Bio-Rad, #1351111) was reconstituted with DMSO and added to each treated sample. One sample was left untreated and unstained to help with instrument setup. Samples were incubated for 30 min at room temperature in the dark before washing in 1 ml PBS. Samples were resuspended in PBS to a concentration of 2 x 10^6 cells/ml.

Cell viability was detected using the ZE5 Cell Analyzer (Bio-Rad, #12004279) using the 355 nm laser in the 447/60 channel. Data were analyzed using FlowJo 10 Software.

Results and Discussion

Plate-Based Comparison of Capture with Protein A vs. Nuvia S Resin

Initial proof of concept studies with parallel scalable purification processes using Protein A affinity or CEX capture purification, performed in 96-well plates, showed better removal of the host cell proteins (HCPs) in a single step with Protein A relative to Nuvia S (Table 1). However, the IEX process produced material that was equally pure after the polish step. This small-scale study partly supports our hypothesis of improving process economics by replacing Protein A resin with an IEX media. This improvement comes not only in terms of the actual difference in resin price, but also the far greater DBC of the Nuvia S Resin (Table 2).

Table 1. Comparison of HCP clearance on 96-well filter plates between Nuvia S Resin and a Protein A resin.

	HCP, ppm Nuvia S Capture	HCP, ppm Protein A Capture
CHO cell supernatant	72,000	90,300
Capture purification	6,800	980
Nuvia Q intermediate polish purification	250	20
Nuvia cPrime final polish purification	5	20

Table 2. Comparison of cost and binding capacity between Nuvia S Resin and a Protein A resin.

	Nuvia S Resin	MabSelect SuRe Protein A Resin
Technique	Strong cation exchange chromatography	Affinity chromatography
Binding capacity	≥110 mg/ml	~35 mg/ml
Cost (25 ml)*	$110	$2,791

* Pricing mentioned is valid for 2018.

Comparable Purity of Biosimilar Antibody Purified with IEX vs. Protein A Resins

The plate-based IEX capture was repeated in 1 ml columns to estimate the levels of purity achieved at a larger scale. Nuvia S is a high-capacity CEX resin (bulletin 5987). The hcDNA contaminants present in the CHO cell extract do not bind to the resin, appearing instead in the flowthrough as shown by the 255 nm absorbance trace (Figure 2A) resulting in a reduction of ~99% hcDNA and ~94% HCP (Table 3). The hcDNA was cleared to below detection level by the intermediate polish purification with Nuvia Q Resin (bulletin 6129). The primary goal of this purification step was to maximize the recovery of the target biosimilar in the flow-through fraction while keeping the highest amount of contaminants bound to the Nuvia Q Column (Figure 2B). We simultaneously saw a decrease of over 99% in the HCP content.

Nuvia cPrime Mixed-Mode Resin was used for final polish purification. It is a hydrophobic cation exchange resin with a functional ligand that can interact with biomolecules not only by electrostatic and hydrophobic interactions, but also by simultaneous hydrogen bonding (bulletin 6242). Nuvia cPrime is tolerant to high salt concentrations in the feedstream due to its hydrophobicity, allowing the loading of the partially purified flow-through sample from the Nuvia Q Column after only one buffer exchange to decrease the pH. The bound biosimilar was eluted by a buffer with slightly higher pH. Both the binding and elution conditions used in this chromatographic step were gentle, which is crucial for maintaining the integrity of the antibody. At the same time, the purification was effective in that a highly pure adalimumab biosimilar was obtained (Figure 2C). Good separation of the monomeric biosimilar antibody from aggregates and other impurities was seen. The impurities were eluted using a buffer with high conductivity. Samples from each step of purification were run on an SDS-PAGE gel to confirm the increase in purity (Figure 3).

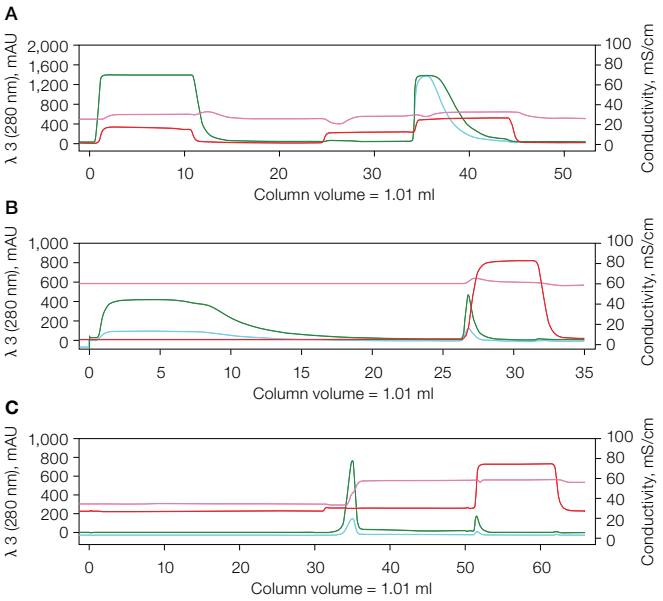


Fig. 2. Chromatograms from purification of the biosimilar. A, capture purification with Nuvia S Resin. The biosimilar obtained from fractions 36 to 44 was subjected to further purification and purity analysis. **B**, intermediate polish purification with Nuvia Q Resin. The column flow-through fractions from 1 to 12 were subjected to further purification and purity analysis. **C**, final polish purification on Nuvia cPrime Resin. The biosimilar eluted from column fractions 33–37 was collected and subjected to purity analysis. λ 2 (255 nm) (—); λ 3 (280 nm) (—); conductivity (—); pH (—).

Table 3. HCP and hcDNA clearance with 1 ml Foresight Columns.

Sample	HCP, ppm	Overall Reduction, %	hcDNA, pg/µl	Overall Reduction, %
CHO supernatant (crude)	170,000	–	26,000	–
Nuvia S Cation Exchange (capture)	10,300	93.91	300	98.86
Nuvia Q Anion Exchange (intermediate polish)	120	99.93	0	100
Nuvia cPrime Mixed-Mode (final polish)	6	99.99	–	–

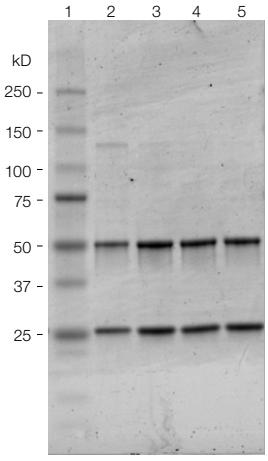
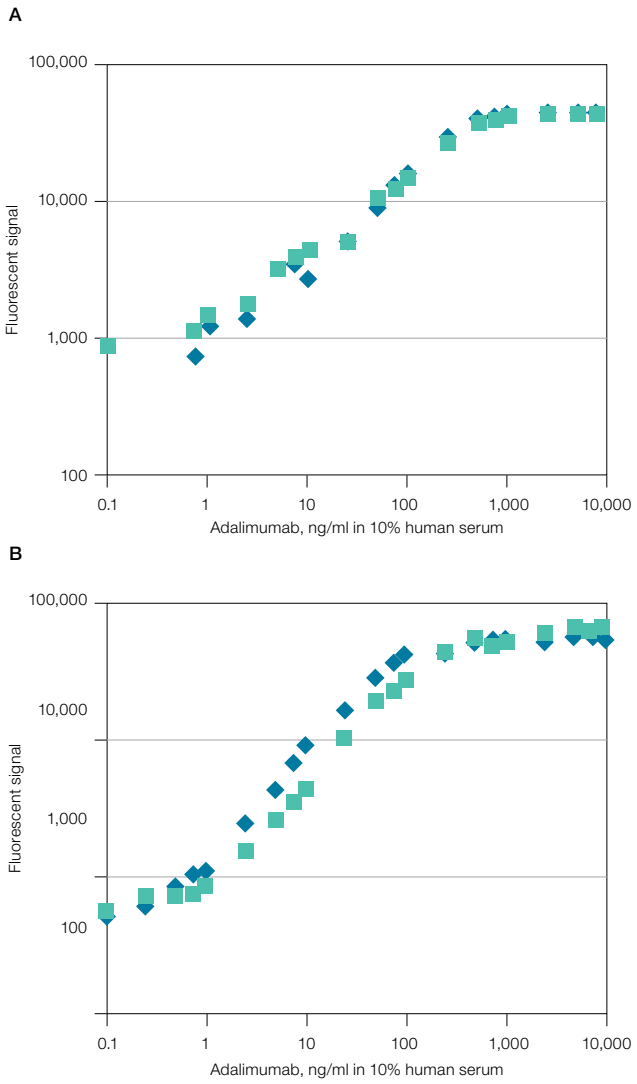


Fig. 3. SDS-PAGE analysis of the chromatographic fractions following purification on Foresight Columns. Lane 1, Precision Plus Protein Unstained Protein Standard; lane 2, CHO supernatant; lane 3, Nuvia S sample; lane 4, Nuvia Q sample; lane 5, Nuvia cPrime sample.

Functional Testing of Purified Biosimilar Binding to the TNFα Ligand Using ELISA and Cell-Based Assays

The integrity of the purified biosimilar antibody was tested using in vitro ELISA and cell-based assays. Part of the extensive characterization that is required by regulatory bodies in filings for biosimilar submissions is to determine the activity of the biosimilar molecule. An in vitro antigen capture ELISA assay showed comparable binding of both the purified adalimumab and the control adalimumab to the TNFα antigen (Figure 4A). The bridging ELISA showed the same trend of binding of both types of adalimumab to an anti-adalimumab antibody (Figure 4B), thereby confirming that the purified HUMIRA biosimilar shows comparable binding patterns to the control antibody. Cell-based assays measuring the ability of the biosimilar to protect cells after the addition of TNFα were carried out. The cell viability seen with the purified adalimumab after addition of TNFα was similar to the one achieved with the control (Figures 4C and 4D), further confirming that the purification protocol did not compromise the functionality of adalimumab.



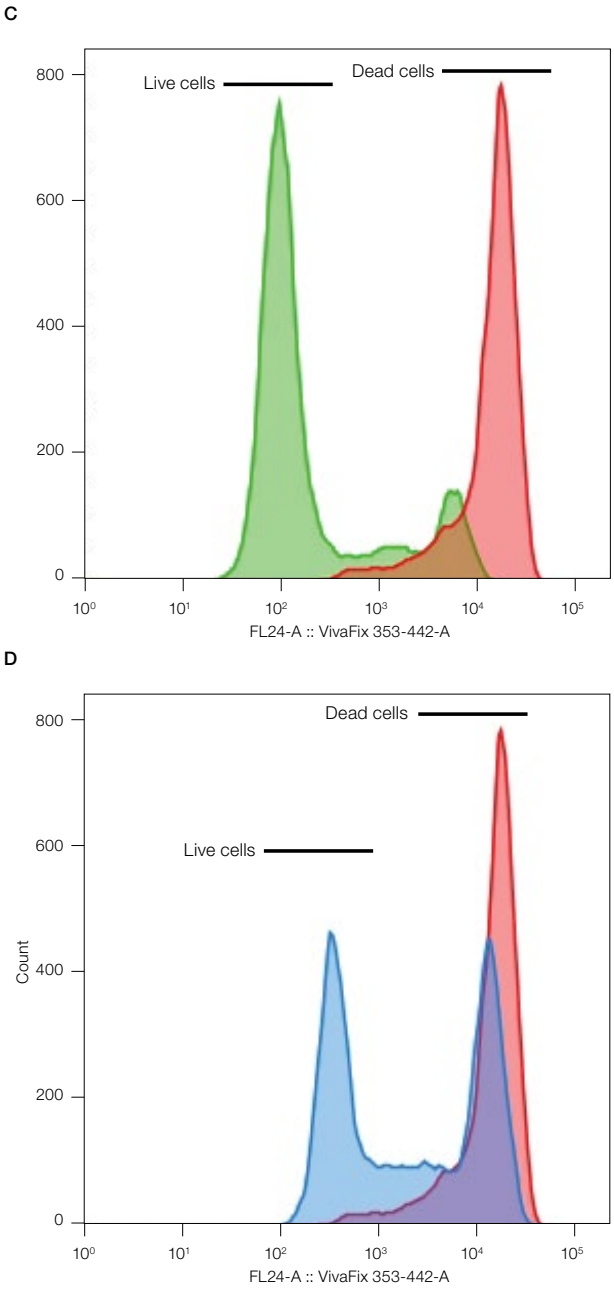


Fig. 4. Functional testing of purified adalimumab. A, pharmacokinetic antigen capture ELISA; comparison of capture of TNFα by the adalimumab standard (◆) and the purified adalimumab (■) in the 96-well plate. **B**, pharmacokinetic bridging ELISA; comparison of anti-adalimumab binding by the adalimumab standard (◆) and the purified adalimumab (■) in the 96-well plate. Both adalimumabs show similar binding trends to TNFα and the anti-adalimumab antibody. **C**, comparison of cells treated with TNFα only (■) and cells treated with TNFα plus adalimumab purified from lysate (PFL) (■). **D**, comparison of cells treated with TNFα only (■) vs. cells treated with TNFα and control anti-TNFα antibody (purchased purified adalimumab) (■). Both adalimumab biosimilars show a protective effect on the cells after treatment with TNFα.

Improved Process Economics Achieved with the IEX-Based Scalable Process

As shown in Figure 5, we decreased the purification costs of the adalimumab biosimilar using a Nuvia S–based purification workflow vs. a Protein A–based one. Even with the cost of an added conditioning step, the Nuvia S workflow improves process economics by reducing resin costs by multiple fold. Numerous further improvements to the process economics of this purification can be made by optimizing the amount of buffer utilized, minimizing feed conditioning, and enabling direct loading between steps.

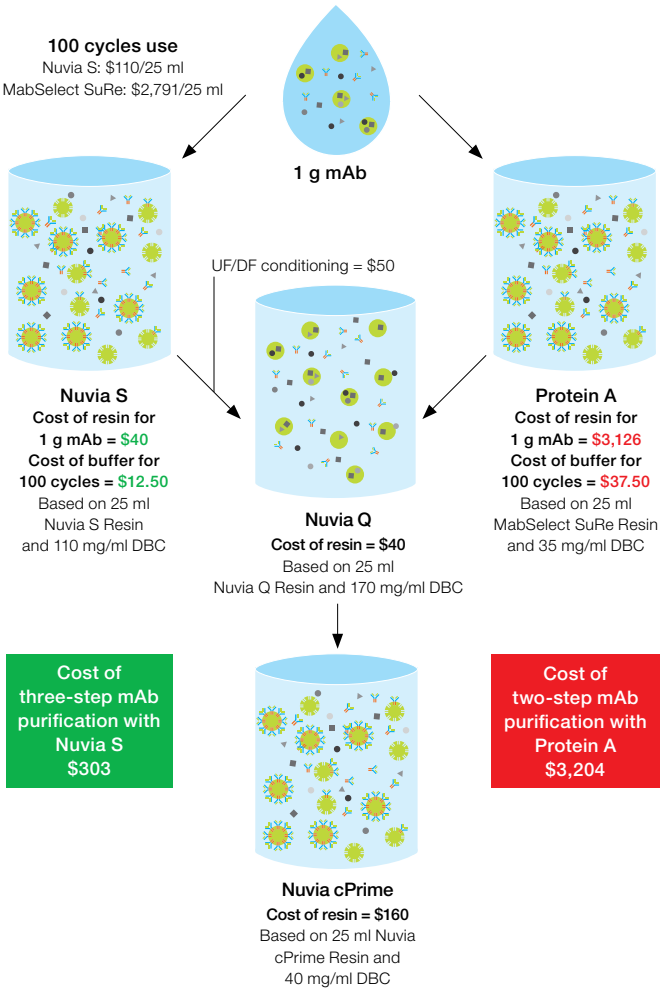


Fig. 5. Cost cutting by replacing the Protein A resin with Nuvia S Resin to improve process economics during biosimilar purification. Pricing mentioned is valid for 2018.

Conclusions

In summary, we show that an IEX and mixed-mode resin–based workflow can deliver a highly pure and active adalimumab biosimilar, equal to that achieved using a purification workflow involving Protein A. The two IEX resins used, Nuvia S and Nuvia Q, purify the target biosimilar efficiently, removing product-related impurities. Use of the Nuvia S cation exchanger for capture led to better process economics, not only by reducing cost but also by enhancing the process speed, a result of the significantly higher binding capacity of the resin. This alternative approach overcomes the issue of purifying any leached Protein A and the need for subsequent bioanalytical assays to monitor for this process impurity. Nuvia S Resin is chemically and mechanically stable and can be used across 100+ purification cycles. In the final purification step, the multiple interaction modes offered by Nuvia cPrime ensured the removal of both product- and process-related impurities. Since all three resins could be operated at high flow rates with low backpressure, this increased the productivity and thus improved the process economics of the workflow. Therefore, an IEX-based purification platform in general, and Nuvia Resin–based purification platforms specifically, have the potential to contribute to faster (to market) and cheaper biosimilars.

References

Blackstone EA and Joseph PF, Jr (2013). The economics of biosimilars. Am Health Drug Benefits 6, 469–478.

DiMasi JA et al. (2016). Innovation in the pharmaceutical industry: New estimates of R&D costs. J Health Econ 47, 20–33.

Gutierrez BJ (2015). Financial analysis of biosimilar development candidates: A case study on the US biosimilar business. dash.harvard.edu/bitstream/handle/1/24078352/GUTIERREZ-THESIS-2015.pdf, accessed September 10, 2018.

Jacoby R et al. (2015). Winning with biosimilars. Opportunities in global markets. deloitte.com/content/dam/Deloitte/us/Documents/life-sciences-health-care/us-lshc-biosimilars-whitepaper-final.pdf, accessed September 10, 2018.

World Health Organization (2009). Expert committee on biological standardization. Guidelines on evaluation of similar biotherapeutic products (SBPs). who.int/biologicals/areas/biological_therapeutics/BIOTHERAPEUTICS_FOR_WEB_22APRIL2010.pdf, accessed September 10, 2018.

BIO-RAD

Bio-Rad Laboratories, Inc.

Life Science Group

Web site *bio-rad.com* ***USA*** *1 800 424 6723* ***Australia*** *61 2 9914 2800* ***Austria*** *43 01 877 89019* ***Belgium*** *32 03 710 53 00* ***Brazil*** *55 11 3065 7550* ***Canada*** *1 905 364 3435* ***China*** *86 21 6169 8500* ***Czech Republic*** *36 01 459 6192* ***Denmark*** *45 04 452 10 00* ***Finland*** *35 08 980 422 00* ***France*** *33 01 479 593 00* ***Germany*** *49 089 3188 4393* ***Hong Kong*** *852 2789 3300* ***Hungary*** *36 01 459 6190* ***India*** *91 124 4029300* ***Israel*** *972 03 963 6050* ***Italy*** *39 02 49486600* ***Japan*** *81 3 6361 7000* ***Korea*** *82 2 3473 4460* ***Mexico*** *52 555 488 7670* ***The Netherlands*** *31 0 318 540 666* ***New Zealand*** *64 9 415 2280* ***Norway*** *47 0 233 841 30* ***Poland*** *36 01 459 6191* ***Portugal*** *351 21 4727717* ***Russia*** *7 495 721 14 04* ***Singapore*** *65 6415 3188* ***South Africa*** *36 01 459 6193* ***Spain*** *34 091 49 06 580* ***Sweden*** *46 08 555 127 00* ***Switzerland*** *41 0617 17 9555* ***Taiwan*** *886 2 2578 7189* ***Thailand*** *66 2 651 8311* ***United Arab Emirates*** *971 4 8187300* ***United Kingdom*** *44 01923 47 1301*

Bulletin 7130 Ver A US/EG *18-0764 1118 Sig 0118*