DESIGNING A NEW PARTICLE TECHNOLOGY AND PH GRADIENT MOBILE PHASE CONCENTRATES FOR ROBUST, HIGH RESOLUTION CHARGE VARIANT ANALYSIS OF MABS

Paula Orens, Matthew A. Lauber, Qi Wang, Susan C. Rzewuski, Hua Yang, Stephan Koza, Mingcheng Xu, Mike F. Morris, Justin McLaughlin, Stephen Shiner and Bei Niu
Waters Corporation, Milford, MA, USA

Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

INTRODUCTION

Ion exchange chromatography (IEX) is routinely relied upon to characterize and monitor the charge variants of protein-based therapeutics such as monoclonal antibodies (mAb),[1] which can very often have implications on drug efficacy and accordingly be flagged as critical quality attributes.[2] There has long been a need to address resolution limitations and challenges related to method implementation and robustness.

A new IEX column technology based on a 3 µm non-porous sorbent, specialized polymerization reactions and a finely tuned sulfonic acid grafting has been developed to improve charge variant analyses of monoclonal antibodies. In addition, a new pH gradient mobile phase system has been developed based on the new particle technology to provide universally applicable cation exchange separations of mAbs having a wide range of pI values.

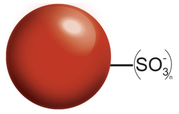


Figure 1. Schematic depicting the particle and surface technology of the BioResolve SCX mAb stationary phase.

***Patent Pending**

METHODS

Panitumumab, infliximab, trastuzumab, adalimumab, and NIST mAb (reference material 8671) were diluted with 18.2 MΩ water to either 5 or 2.5 mg/mL. The mAb Charge Variant Standard (p/n 186009065) was reconstituted with 100 µL of 18.2 MΩ water. Analyses were performed using an ACQUITY UPLC H-Class Bio and ACQUITY UPLC TUV detector. Mab separations were performed on a 3µm, 4.6 x 50 mm BioResolve SCX mAb column. Analyses were performed with UV detection at 280 nm using Empower 3.

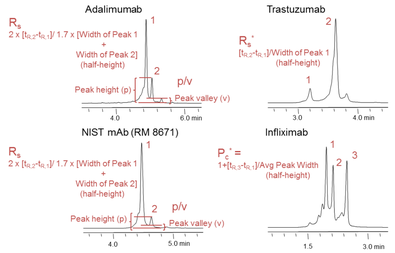


Figure 2. Example Calculations and Data Analysis.

RESULTS AND DISCUSSION

Base Particle Morphology, Size and Composition

Numerous insights have been made about what makes for the best optimization of an ion exchange sorbent.

Two prototype stationary phases were prepared with comparable ionic capacity, their only difference being particle diameter.

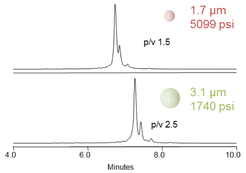


Figure 3. Salt gradient separations and UV chromatograms of adalimumab as obtained with early 2.1 x 50 mm IEX prototype columns packed with either a sub 2-µm or a 3.1 µm stationary phase.

It was preferred to pursue the development of an approximately 3 µm non-porous stationary phase which appears to be more optimal for mAb separations and facilitate separations on multiple LC platforms.

This 3 µm non-porous stationary phase's capacity to withstand excess pressure was essential for ensuring column bed stability.

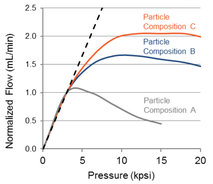


Figure 4. Mechanical strength testing of non-porous base particles of varying chemical composition. Specialized columns were flushed with methanol at increasingly higher pressures and the resulting flow rates were measured. Deviations from the theoretical flow rate (dashed line) indicate perturbation of either the particles and/or the packed bed.

Composition C represents the newly designed base particle and its amenability to relatively high flow rate method conditions.

Tailoring a Surface Chemistry for mAbs

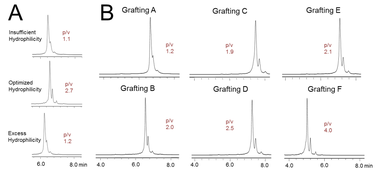


Figure 5. Salt gradient separations and UV chromatograms of adalimumab as obtained with 4.6 x 50 mm IEX prototype columns packed with stationary phase prepared with (A) varying levels of hydrophilicity and (B) varying compositions of sulfonic acid grafting.

In sum, each of the above optimizations forms the basis of the new BioResolve SCX mAb stationary phase, which is best summarized as a 3 µm non-porous sorbent made from specialized polymerization reactions and a finely tuned sulfonic acid grafting.

Performance, Robustness and Lifetime

The BioResolve SCX mAb column showed higher resolving power.

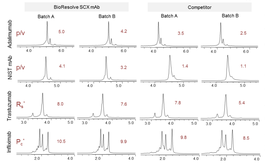


Figure 6. Salt gradient separations of four mAbs as performed with 4.6 x 50 mm BioResolve SCX mAb columns or competitor 4.0 x 50 mm SCX 3 µm columns packed with unique batches of stationary phase.

Similar testing was performed at an even broader scale to assess the reproducibility of 7 different batches of BioResolve SCX mAb stationary phase.

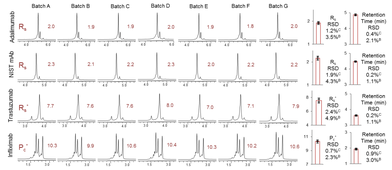


Figure 7. Reproducibility of the BioResolve SCX mAb stationary phase. UV chromatograms collected using 4.6 x 50 mm BioResolve SCX mAb columns packed with 7 unique batches of stationary phase. Average resolution metrics and retention time data shown along with RSD values collected from either 7 columns packed with the same stationary phase batch (column-to-column variability)[C] or 7 columns packed with 7 unique batches of stationary phase (batch-to-batch variability)[B].

High confidence can be placed in a BioResolve SCX mAb column to know it can produce both high resolution and the reproducibility.

Loadability is important since charge variant separations are used to acquire fractions for structure function studies.

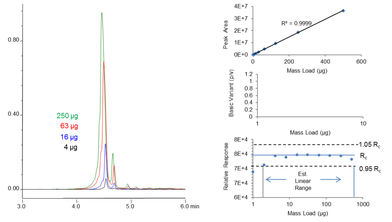
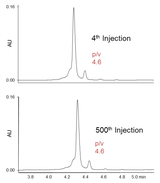


Figure 8. Loading capacity of a 4.6 x 50 mm BioResolve SCX mAb column as studied with increasing mass loads of NIST mAb (RM 8671).

Across this range of mass loads, excellent peak area linearity was observed (R^2 = 0.9999) and only a slight decrease in resolution was encountered at the higher end mass loads of 250 and 500 µg. This column technology should thereby lend itself to applications requiring elevated mass loads and fractionation. Based on these data, it is likely for a 4.6 mm ID column to maintain noteworthy resolution up to 1 or even 2 mg mass loads depending on sample properties.

A 4.6 x 50 mm BioResolve SCX mAb column was also subjected to lifetime testing.



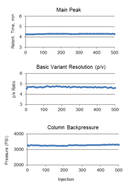


Figure 9. Lifetime testing of a BioResolve SCX mAb Column using a salt gradient separation where repeat separations of NIST mAb were performed for 500 injections.

Consistent column performance can be confirmed by the fact that retention times, resolution and pressure were essentially unchanged

Development of a Platform pH Gradient Method for mAb Charge Variant Analysis

The development of BioResolve CX pH Concentrates was based both on theoretical predictions and empirical optimization according to observations relating mobile phase composition to resolving power.

Separations achieved with the finely tuned compositions of the BioResolve CX pH Concentrates are portrayed in Figure 10A.

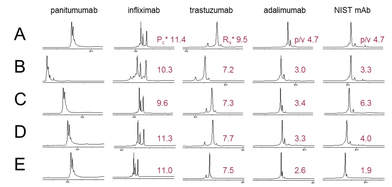


Figure 10. Optimization of pH gradient mAb charge variant separations using mobile phases composed of different buffer salts.

pH Linearity and Kinetic Effects of BioResolve CX pH Concentrates

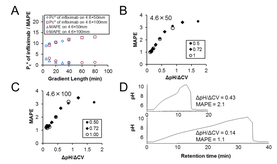


Figure 11. The relationship between resolution, pH linearity and gradient steepness for pH gradient separations with BioResolve CX pH Concentrates.

Peak resolution improves with increasing gradient times. It was observed that pH linearity improved with increasing gradient time. Similar trends of MAPE versus ΔpH/Δcolumn volume (CV) were observed with columns of differing dimensions, including 2.1 versus 4.6 mm IDs and 50 versus 100 mm lengths. To achieve high precision pH traces, it is recommended that a gradient steepness (ΔpH/ΔCV) of 0.5 or lower be employed. Hence, a potential method condition to consider for a 4.6×50 mm column entails a flow rate of 1.00 mL/min across a 30 min 0-100%B gradient.

Performance, Robustness and Lifetime of BioResolve CX pH Concentrates

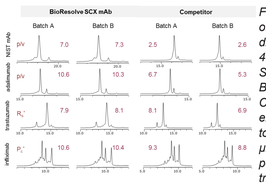


Figure 12. Separations of mAbs performed with different batches of 4.6×50 mm BioResolve SCX mAb columns and BioResolve CX pH Concentrates or different batches of competitor 4.0×50 mm SCX 3 µm columns and competitor CX pH Concentrates.

At the same pH gradient slope of 0.17 pH unit/minute, the BioResolve separation technologies showed higher resolving power than the leading alternative technologies. Sizeable gains in resolution were achieved for high pI mAbs, e.g. adalimumab and NIST mAb, with the BioResolve separation technologies.

The combination of BioResolve CX pH Concentrates and BioResolve SCX mAb column facilitates faster analysis versus legacy approaches to LC-based charge variant profiling.

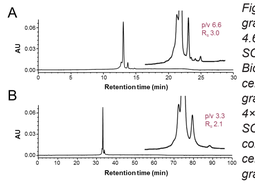


Figure 13. UV chromatogram of NIST mAb on a 4.6×50 mm BioResolve SCX mAb column using BioResolve CX pH Concentrates with a 20-minute gradient (A) versus a 4×250 mm competitor SCX 10 µm column with competitor CX buffer concentrates with a 50-minute gradient (B).

Superior peak resolution was achieved with the BioResolve separation technologies with a 20-minute gradient versus the competitor setup with a 50-minute gradient. With the same sample load, not only were the major basic variants better resolved from the main peak, but three minor basic variants were also well resolved with the BioResolve separation technologies.

Column lifetime testing was performed with BioResolve CX pH Concentrates to examine the robustness of BioResolve SCX mAb columns with pH gradient separations.

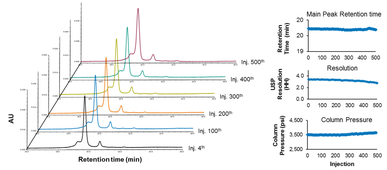


Figure 14. Lifetime testing of a BioResolve SCX mAb Column using BioResolve CX pH Concentrates.

No significant difference was observed in the representative chromatograms of NIST mAb throughout the 500 injections. Moreover, consistent retention times and column pressure were observed across the 500 injections, and the resolution of the basic variant only drifted slightly toward the end of the lifetime test.

Method Optimization to Improve Throughput and/or Resolution with BioResolve CX pH Concentrates

BioResolve CX pH Concentrates provide robust and high resolution separations of mAbs with pI values ranging from 6 to 10. To further improve throughput and/or resolution, as little as two injections with a generic 0-100%B gradient method are needed.

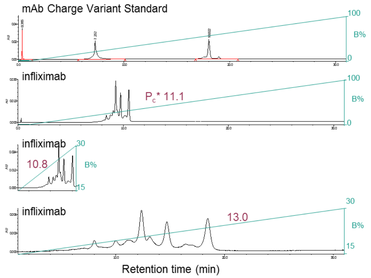


Figure 15. Method optimization to improve throughput or resolution for a particular mAb. Representative chromatograms of the Waters mAb Charge Variant Standard (A) and infliximab (B) on a 4.6×50 mm BioResolve SCX mAb column using BioResolve CX pH Concentrates with a generic gradient of 0-100% B at a pH gradient slope of 0.17 pH unit/minute, a shortened gradient at a slope of 0.17 pH unit/minute (C), or a focused gradient at a slope of 0.026 pH unit/minute (D) at a flow rate of 1.00 mL/min.

CONCLUSION

- **A new IEX column technology based on a 3 µm non-porous sorbent, specialized polymerization reactions and a finely tuned sulfonic acid grafting has been developed to improve charge variant analyses of monoclonal antibodies.**
- **Its performance has been confirmed to be universally applicable to multiple mAbs, and it has been proven to be reproducible and robust by way of observations on minimal column-to-column and batch-to-batch variation as well as column lifetime and loadability.**
- **A new pH gradient mobile phase system, based on BioResolve CX pH Concentrates, has been developed to provide universally applicable cation exchange separations of mAbs having a wide range of pI values.**
- **The performance of this method has been confirmed to be reproducible from batch-to-batch and to afford column lifetimes up to and beyond 500 injections.**

References

1. Fekete, S., Beck, A., Veuthey, J. L. & Guillarme, D. Ion-exchange chromatography for the characterization of biopharmaceuticals. Journal of pharmaceutical and biomedical analysis 2015, 113, 43-55.
2. Auclair, J. R.; Rathore, A.; Krull, I., Charge-Variant Profiling of Biopharmaceuticals. LCGC 2018, 36 (1).

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS