Waters™

Nota de aplicación

# Designing High-Pressure Capable, Corrosion-Resistant Columns for Cation-Exchange Chromatography

Mathew Delano, Steve Shiner, Matthew A. Lauber, Justin McLaughlin, Steven Byrd, Susan C. Rzewuski

Waters Corporation

This is an Application Brief and does not contain a detailed Experimental section.

## Abstract

This Application brief demonstrates Mitigate frit and column hardware corrosion, and prevent metal cation poisoning with BioResolve SCX mAb Columns.

## Benefits

BioResolve SCX mAb Column hardware is purposely designed to mitigate corrosion while maintaining packed column performance.

## Introduction

Cation-exchange chromatography is a common technique used for analyzing monoclonal antibody (mAb) biotherapeutics for charge variants. Successful chromatography involves using a gradient of either increasing ionic strength, or a simultaneous change in pH and ionic strength of the mobile phase in order to cleave the mAb charge variants from the negative surface of the cation-exchange column. As such, it is important to maintain the number of negatively charged adsorption sites on the cation-exchange-stationary-phase particles contained

within a column.

Metals present in the chromatography system have the potential to corrode in salt-containing mobile phases, especially at high and low pH values. While use of conventional stainless steel grades are adequate for use in LC separations that do not contain salt (e.g., reversed-phase LC), use of stainless steel can be problematic even when the salt in the separation eluent is used at neutral pH (e.g., 7.0). In such a situation, the susceptible metal will corrode and release divalent and trivalent positively charged cations, which will bind strongly to a cation-exchange stationary phase. This process results in decreased and varying number of negatively charged, cation adsorption sites, causing problems that can include changes in analyte retentivity, peak shape, and ability to separate species with minor charge differences.

## Results and Discussion

The frit and column hardware used in the BioResolve SCX mAb Column (Figure 1) as well as the VanGuard FIT (Fully Integrated Technology) option have been purposely designed to mitigate corrosion, while maintaining packed column performance. Both construction materials and design implementation were considered to accomplish this goal. Most notably, titanium is used along with polymeric seals in strategic locations to afford excellent performance despite regular exposure to corrosive conditions.

To demonstrate this corrosion resistance, the BioResolve SCX mAb Column hardware was subjected to extreme test conditions. Accelerated corrosion testing with 1 M sodium chloride, pH 7 shows a drastic difference between BioResolve SCX hardware and standard stainless steel hardware.

Figure 1. Explosion view of BioResolve SCX mAb Column hardware.

Both hardware types were exposed to 1 M sodium chloride at pH 7 for seven days at room temperature. At the conclusion of the seven-day storage, the endnuts of each column were removed and the frits were examined.

The standard hardware showed visual rusting in as little as one week, whereas the BioResolve design showed none. Results of this testing are shown in Figure 2.

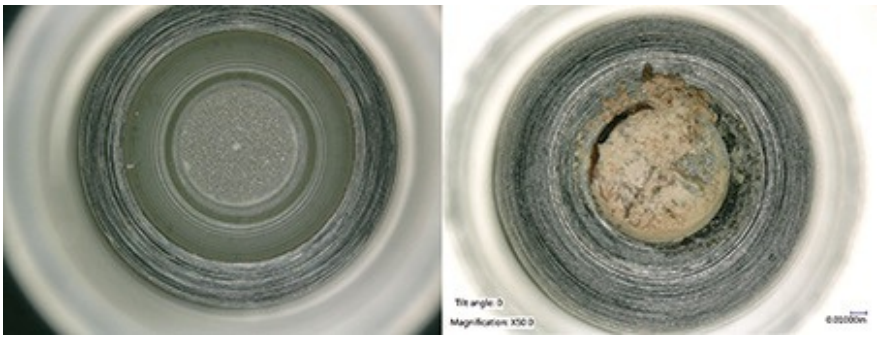

Figure 2. Accelerated corrosion testing of 2.1 mm column frits. BioResolve SCX mAb hardware is shown on the left and standard hardware on the right.

To address other potential concerns, BioResolve SCX mAb Columns have also been subjected to shelf life testing, wherein columns are used with halide salt mobile phases, purged to 20% methanol storage solvent, and stored for one month before being applied again to a separation. Figure 3 displays chromatograms collected across such a study. As can be seen, a separation of NIST mAb (NIST reference material 8671) was as effective after storage as it was before. Half height resolution from a salt gradient separation of the molecule was observed to be 2.42 and 2.54 before and after storage, respectively.

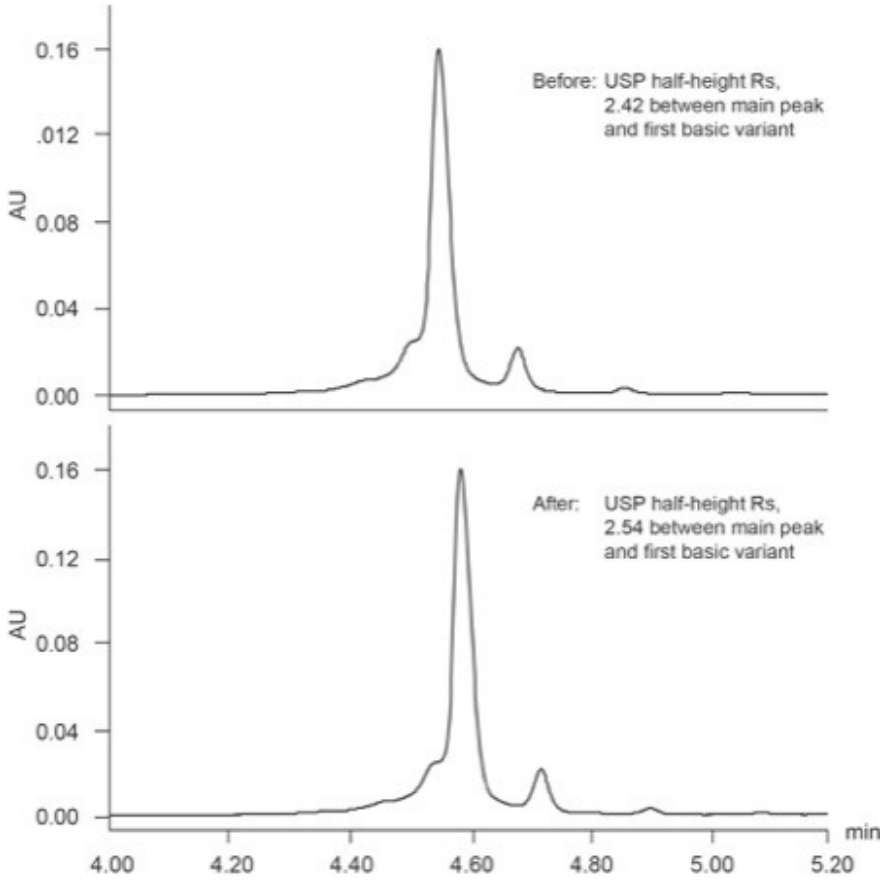


Figure 3. Shelf life testing of a BioResolve SCX mAb, 4.6 x 50 mm Column (p/n: 186009058) as studied with an ACQUITY UPLC H-Class Bio System, flow rate of 0.96 mL/min, and 7.5 minute salt gradient between 10 and 200 mM NaCl (20 mM MES, pH 6.0).

In addition to this one-month study, an eight-month study was completed with a prototype iteration of the stationary phase. Columns were packed, tested with salt gradients, flushed with 20% methanol storage solvent, and retested and autopsied after eight months. No corrosion was observed over this eight-month period.

The corrosion resistance and amenability of BioResolve SCX mAb Column hardware to efficient packing processes has unlocked new levels of performance for ion-exchange chromatography. Accordingly, there is significant opportunity to heighten the analytical capabilities of mAb charge variant profiling without a need for concern of corrosion and its related interferences.

## Conclusion

Cation-exchange chromatography is frequently used to characterize biotherapeutics using eluents that can result in corrosion of the stainless steel frit and column hardware commonly used. This study demonstrates that the use of titanium and polymeric seals in the BioResolve SCX mAb Column and optional VanGuard FIT offerings overcome these concerns.

## Featured Products

- ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>

720006501, March 2019