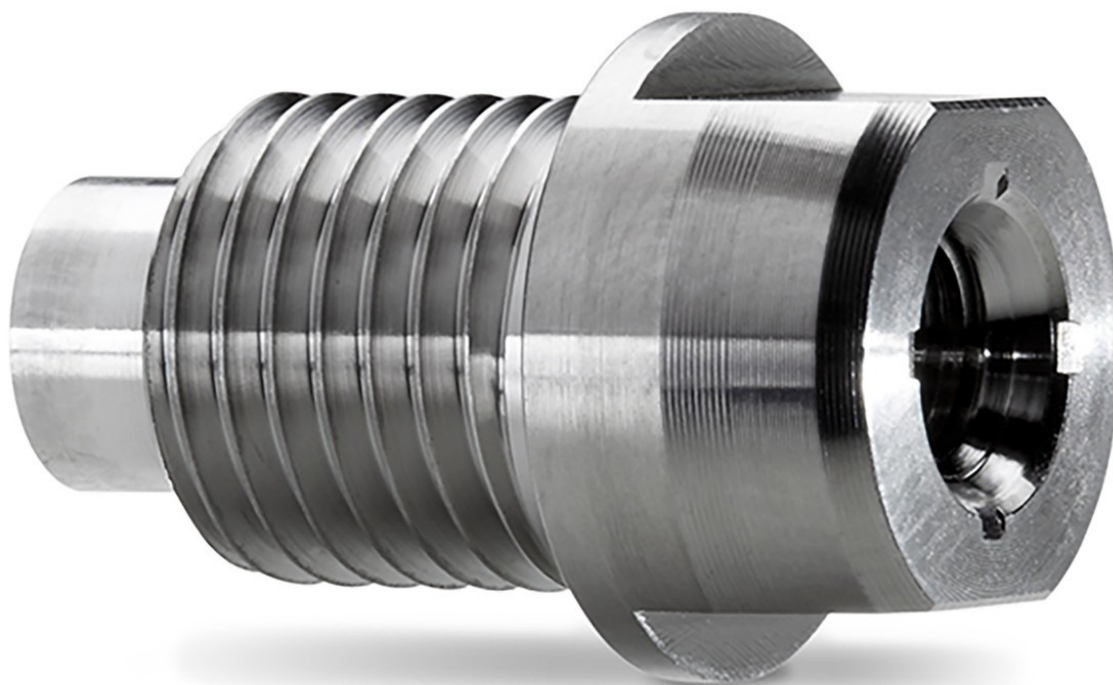


VanGuard FIT: A Breakthrough in Guard Column Performance for Challenging Chromatographic Separations

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

Abstract

This Application brief describes to extend analytical column lifetimes by preventing particulate and chemical fouling, without the deleterious effects to resolution that is commonly associated with using guard columns.

Benefits

VanGuard FIT represents a more purposeful approach to guard column design and functionality, providing unprecedented ease-of-use in an ultra-low dispersion format.

Introduction

One of the most prevalent failure modes of chromatography columns is the build-up of particulates or other column fouling compounds at the head of the column. This is especially true in UPLC applications where stationary phase particles are very small, making the packed bed an incredibly fine filter. There are a number of sources from where such contamination can derive, but often it is due to the presence of microbes, buffer precipitates, sample related particulates, or sample matrices. A common practice to prevent analytical column fouling is to employ a guard column. However, this approach typically causes a reduction in resolving power due to added extra-column dispersion. Less sensitive applications may not be significantly affected by the addition of a guard, but many others, including emerging bio-separations applications, rely on accessing as much of a column's resolving power as possible. In these situations, there is little tolerance to anything that could reduce a column's performance. Guard columns are less attractive in such cases, forcing the user to live with short column lifetimes and higher operating costs.

Results and Discussion

Guard column geometry, additional ferrule connections, and improper installation are all contributors to extra-column dispersion when restricted to traditional designs. Each of these problems has been addressed with VanGuard FIT* (Fully Integrated Technology). **Patent pending*

The VanGuard FIT represents a more purposeful approach to guard column design and functionality, providing

unprecedented ease-of-use coupled with an ultra-low dispersion format. By removing extraneous tubing and ferrule connections between the guard and the analytical column, sound connections can be made easily and reproducibly, and extra-column volume is considerably reduced (Figure 1). This translates to optimal performance, for even the most critical separations.

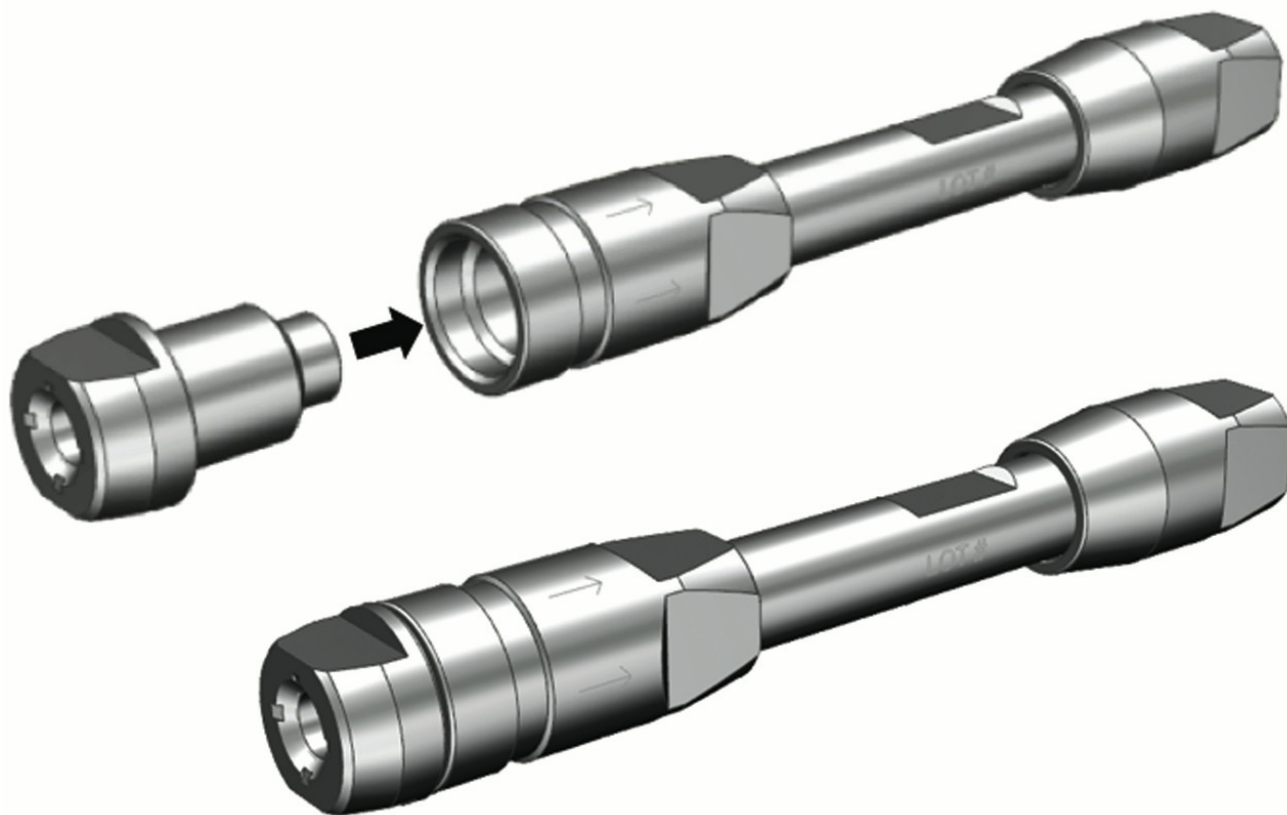


Figure 1. BioResolve SCX mAb, 4.6 x 50 mm Column with integrated VanGuard FIT. Replaceable cartridge screws directly into the column inlet end nut. Optimal installation has never been easier nor more efficient in terms of maintaining analytical columns' resolving power.

Resolution is virtually unaffected by the addition of the VanGuard FIT. To demonstrate this, optimized gradients of increasing ionic strength were performed to generate charge variant profiles¹ for NISTmAb (NIST RM 8671) using BioResolve SCX mAb Columns with and without the VanGuard FIT offering. Figure 2 shows that resolution achieved between the main peak (peak 1) and primary basic isoform (peak 2) of NISTmAb was not influenced by the use of a column equipped with VanGuard FIT.

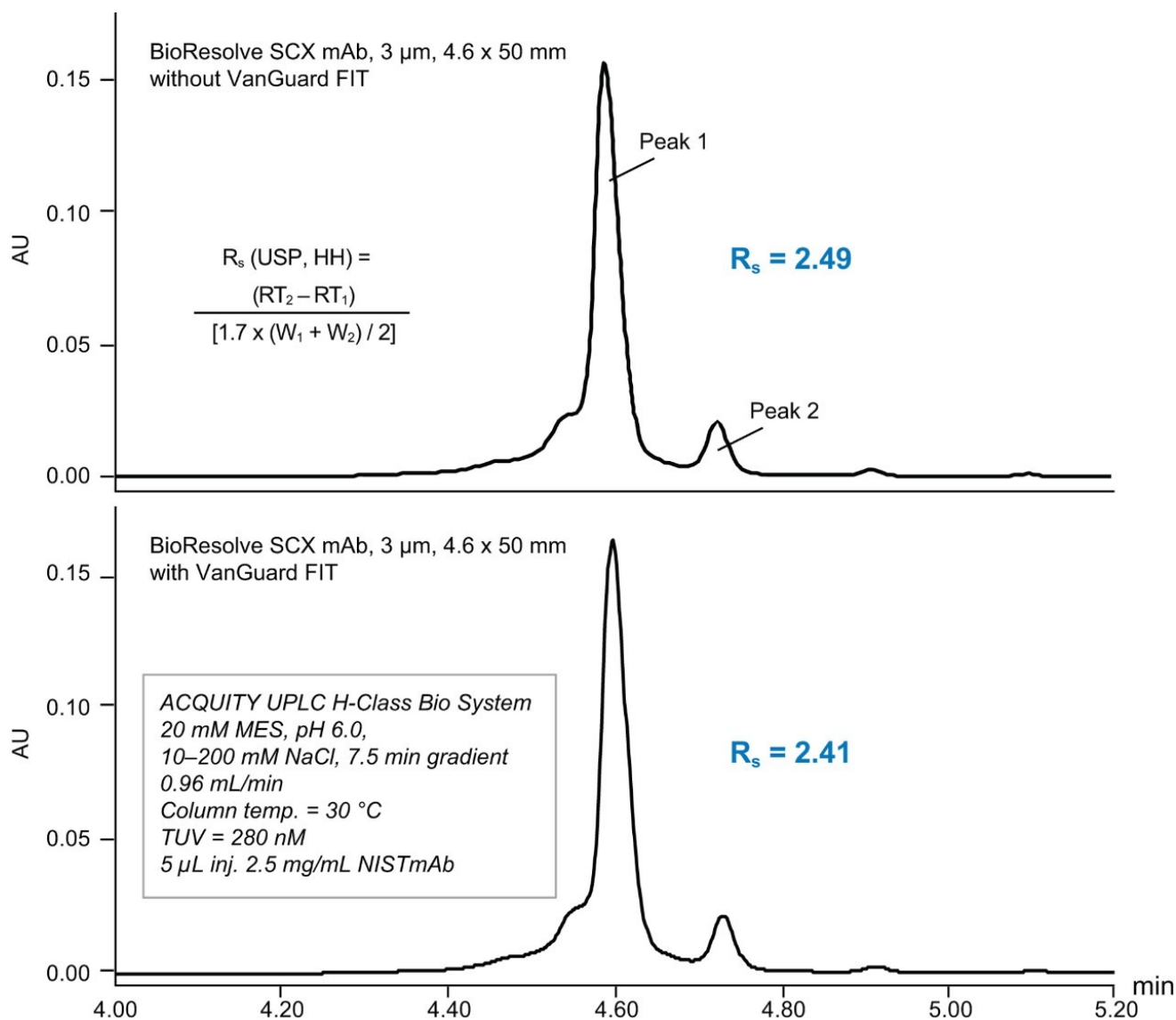


Figure 2. Charge variant profiles¹ for NISTmAb. No significant differences in chromatographic performance on BioResolve SCX mAb Columns without (top; p/n: 186009058) or with (bottom; p/n: 186009057) VanGuard FIT. It has also been evaluated that the VanGuard FIT serves its purpose. The trapping effectiveness of the VanGuard FIT was demonstrated in two ways. First, a suspension of 0.1 μ m polystyrene beads was repeatedly injected onto a BioResolve SCX mAb, 4.6 x 50 mm Column with VanGuard FIT (p/n: 186009057) until a 30% backpressure increase was realized. The VanGuard FIT was then replaced, and the column was observed to return to normal. This process was repeated to further emphasize trapping effectiveness (Figure 3A).

Next, a BioResolve SCX mAb, 4.6 x 50 mm Column with VanGuard FIT was subjected to alternating injections and salt gradient separations of rat plasma and NISTmAb until a significant decrease in resolution between the main peak and first basic isoform of NISTmAb was observed. Figure 3B shows the degradation in performance

due to this chemical fouling as well as the recovery of column performance upon replacement of the VanGuard FIT Cartridge.

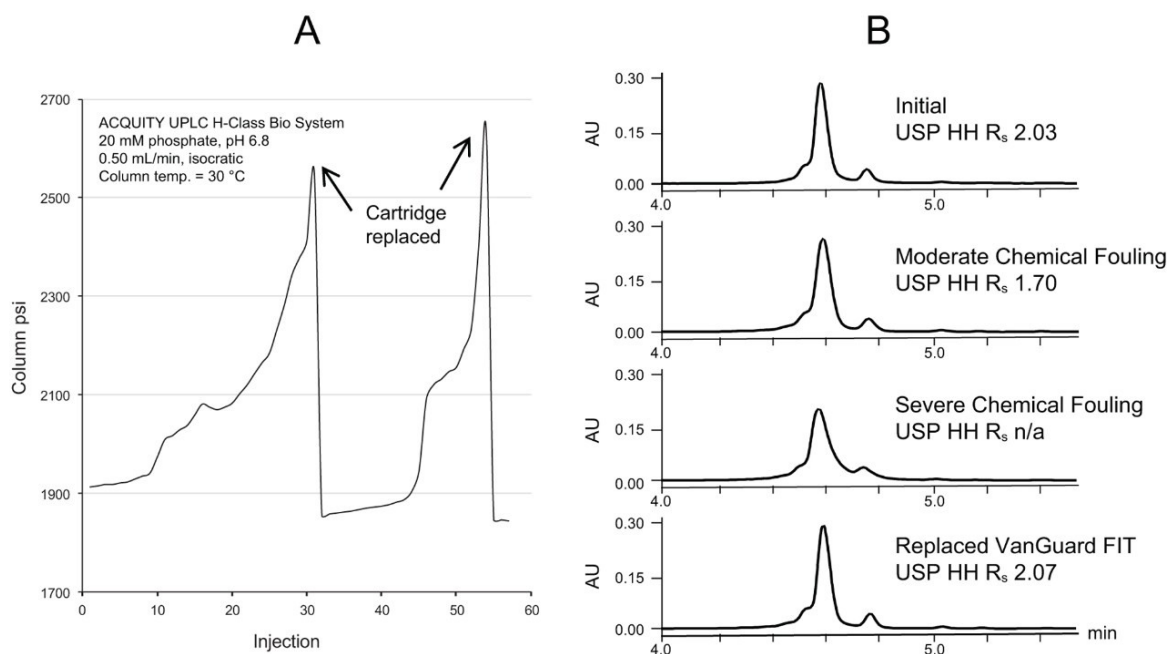


Figure 3. (A) A suspension of 0.1 μm polystyrene beads was repeatedly injected on a BioResolve SCX mAb, 3 μm , 4.6 x 50 mm Column until $\sim 30\%$ backpressure increase was realized. Backpressure returned to normal upon replacement of the VanGuard FIT. (B) Rat plasma was injected into salt gradients (ACQUITY UPLC H-Class Bio System, 20 mM MES, pH 7.0, 10 min gradient, 10 to 200 mM NaCl, 30 °C) until loss of resolution was observed. Resolution was recovered upon VanGuard FIT replacement.

Conclusion

The VanGuard FIT Cartridge design enables chromatographers to significantly extend the life of their analytical column, while maintaining optimal resolution for the most challenging separations. In the examples shown, the VanGuard FIT Cartridge protected BioResolve SCX mAb Columns in extreme cases of both particulate and chemical fouling. When backpressure increase or resolution decrease became excessive due to fouling, the VanGuard FIT Cartridge was replaced and column performance was recovered. Replacement was made quickly and easily, maximizing analytical column lifetime and minimizing down time. In the hands of users, there is now more opportunity than ever to preserve chromatographic columns and their resolving power.

Note: VanGuard FIT Cartridges (2.1 x 5 mm or 3.9 x 5 mm) can only be used on BioResolve SCX mAb Columns containing the VanGuard FIT option.

References

1. Auclair, J. R.; Rathore, A.; Krull, I. Charge-Variant Profiling of Biopharmaceuticals. *LCGC*. 2018, 36 (1), 26–36.

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