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Applikationsbericht

Extending Column Lifetime using VanGuard™ Fully Integrated Technology (FIT) Column Protection

Kenneth D. Berthelette, Bonnie A. Alden, Donna Osterman, Maureen DeLoffi, Jonathan E. Turner

Waters Corporation

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

Abstract

Analysis of compounds present in matrix is a common workflow in many industries and includes but is not limited to food and environmental testing, bioanalysis, and forensics, metabolomics, and pharmaceuticals. The presence of excipients like fillers and binding agents in formulated products can foul an analytical liquid chromatography (LC) column after just a few repetitive injections leading to poor chromatographic results and in some cases, a failed assay. With proper sample pre-treatment a column can last longer, however not all assays are amenable to techniques such as solid phase extraction (SPE) or sample filtration. Apart from sample pre-treatment, a common method to extend column lifetime is to use a guard column or guard cartridge which can be replaced regularly. The guard device is designed to capture chemical and/or particulate components before they can reach and damage the analytical column. In this work a standard unguarded column configuration was compared to the VanGuard Fully Integrated Technology or FIT column configuration for the analysis of spiked infant formula. The VanGuard FIT Column configuration provided longer column lifetimes when the cartridges were replaced at regular intervals.

Benefits

- Increased column lifetime by using VanGuard FIT columns
- Routine replacement of FIT cartridges maintains the column performance achieved with neat standard solutions
- Improved ease of use of VanGuard FIT columns compared to traditional guard columns and holders

Introduction

Column fouling is a common cause for decreased column lifetime, poor analytical results, and loss of productivity. Often, fouling is caused by matrix components, whether they be excipients like fillers or endogenous compounds like phospholipids, which can precipitate out of solution or get stuck inside the frits and pores of analytical columns. For some analyses, these components can be removed via proper sample preparation or developing an appropriate method for analysis. Solid phase extraction or filtration are the most common methods of sample pre-treatment to remove potentially harmful components. However, for some assays sample preparation is not suitable as it introduces variability or removes important compounds that need to be analyzed. In those cases, using a guard column or guard cartridge to protect the analytical column is a prudent measure to ensure proper column performance over time.

Guard devices are typically short, <20 mm, columns or cartridges which are installed upstream of the analytical column in an LC system. This column or cartridge is the first to experience matrix compounds and will foul first during routine testing. Unlike analytical columns, guard columns are meant to be changed when they become fouled. Routine replacement of guard columns or cartridges protects the analytical column from the potentially problematic matrix components, increasing column lifetime.[1–2] Additionally, guard cartridges and columns are generally less expensive than the analytical column, which makes replacing them better financially. The MaxPeak™ Premier VanGuard FIT Column configuration introduces a newer and more user-friendly approach to using guard columns. The VanGuard FIT columns feature a fully integrated guard cartridge at the inlet of the column. VanGuard FIT columns provide the same level of protection for analytical columns as traditional guard devices using an all-in-one design. The work shown here demonstrates how the VanGuard FIT columns work when used to analyze a suspended matrix sample spiked with analytes.

Experimental

Sample Description

Two samples were created for testing. First a neat standard mixture of 2-acetylfuran, acetanilide, acetophenone, propiophenone, butylparaben, and valerophenone at 4 µg/mL each in 10:90 acetonitrile: water was created. This mixture was used to measure column efficiency in the absence of matrix. The challenge sample was created by combining 75 mL of the above mixture with 250 µL of over-the-counter infant formula, prepared as described in the instructions.

Method Conditions

LC Conditions

LC systems:	ACQUITY™ UPLC™ I-Class PLUS with Binary Solvent Manager (BSM), Sample Manager-Fixed Loop (SM-FL), and PDA detector
Detection:	UV @ 254 nm
Column(s):	ACQUITY Premier BEH™ C_{18}, 2.1 x 100 mm, 1.7µm (p/n: 186009453) ACQUITY Premier BEH C_{18}, 2.1 x 100 mm VanGuard FIT, 1.7 µm (p/n: 186009457)
Column temp.:	40 °C
Sample temp.:	10 °C
Injection volume:	5.0 µL

Flow rate:	0.6 mL/min
Mobile phase A:	Milli-Q Water
Mobile phase B:	Acetonitrile
Gradient profile:	Isocratic hold at 10% B for 0.25 minutes, linear ramp to 95% B in 2.25 minutes. Hold for 0.1 minutes and re-equilibrate column at 10%B for 3.0 minutes. Total cycle time 6 minutes.

Data Management

Chromatography software	Empower™ 3 SR2

Results and Discussion

The VanGuard FIT Column configuration is a new and innovative fully integrated guard design for analytical columns. Unlike previous guard designs, the VanGuard FIT Column has the guard cartridge fully assembled into the column's inlet, as opposed to a being a separate assembly/device. The VanGuard Cartridge is directly threaded into the inlet of the VanGuard FIT Column without the need for tubing and ferrules, which reduces band broadening and improves performance. Other designs of guard columns, Figure 1A, have additional connections that need to be made to install the guard column, or guard cartridge holder. These connections are prone to leaks if not installed correctly. The VanGuard FIT columns however, Figure 1B, do not have that added connection reducing that issue. Both VanGuard FIT columns, and the VanGuard Cartridge Holder use the same design for the actual guard cartridge, Figure 1C, which screws into the appropriate holder.

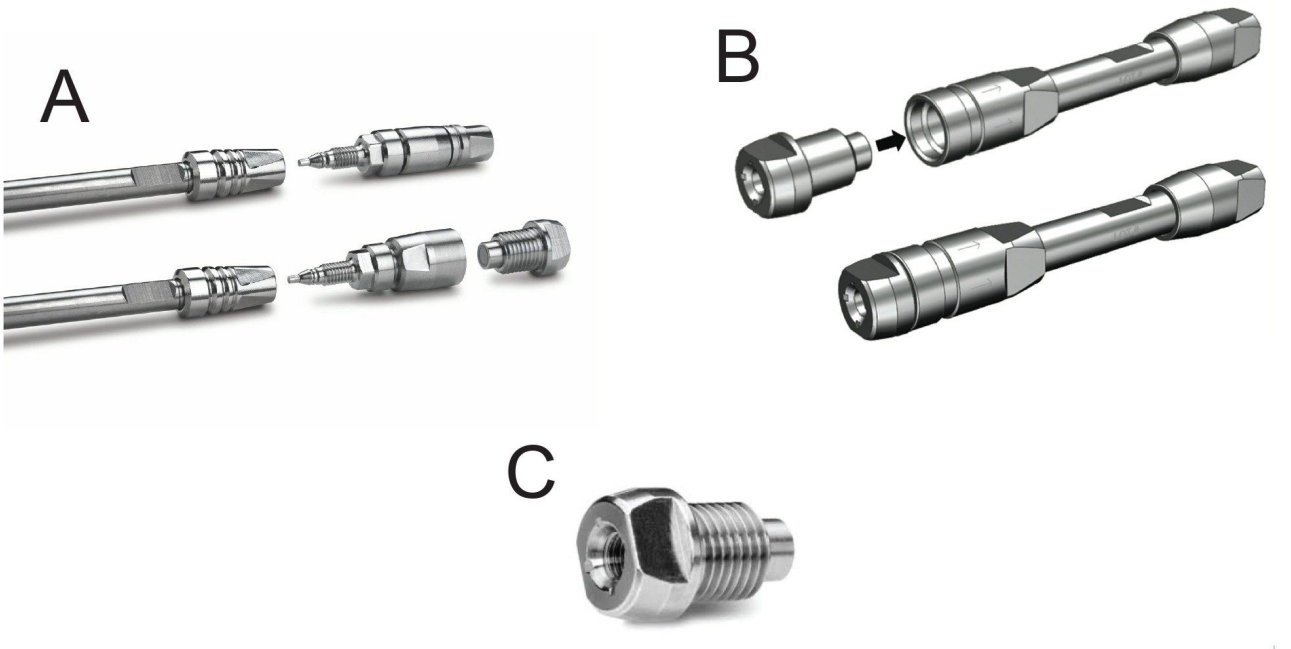

Figure 1. Guard column and cartridge designs.

A. Non-integrated designs using cartridge holders or fully packed guard columns,

B. VanGuard FIT integrated cartridge holder design,

C. guard cartridge design.

Beyond the ease-of-use updates that were implemented for VanGuard FIT columns, the design still protects the analytical column from matrix components, assuming the cartridge is changed regularly. When implementing guards for the first time determining how frequently the cartridge should be changed to maintain column performance is required. In the work performed, two standard ACQUITY Premier BEH C_{18} columns were used to analyze spiked infant formula. During testing the peak capacity using peak width at 13.4% peak height was monitored and plotted. Figure 2 shows a plot of percentage of peak capacity for both columns compared to a third column analyzing neat standard.

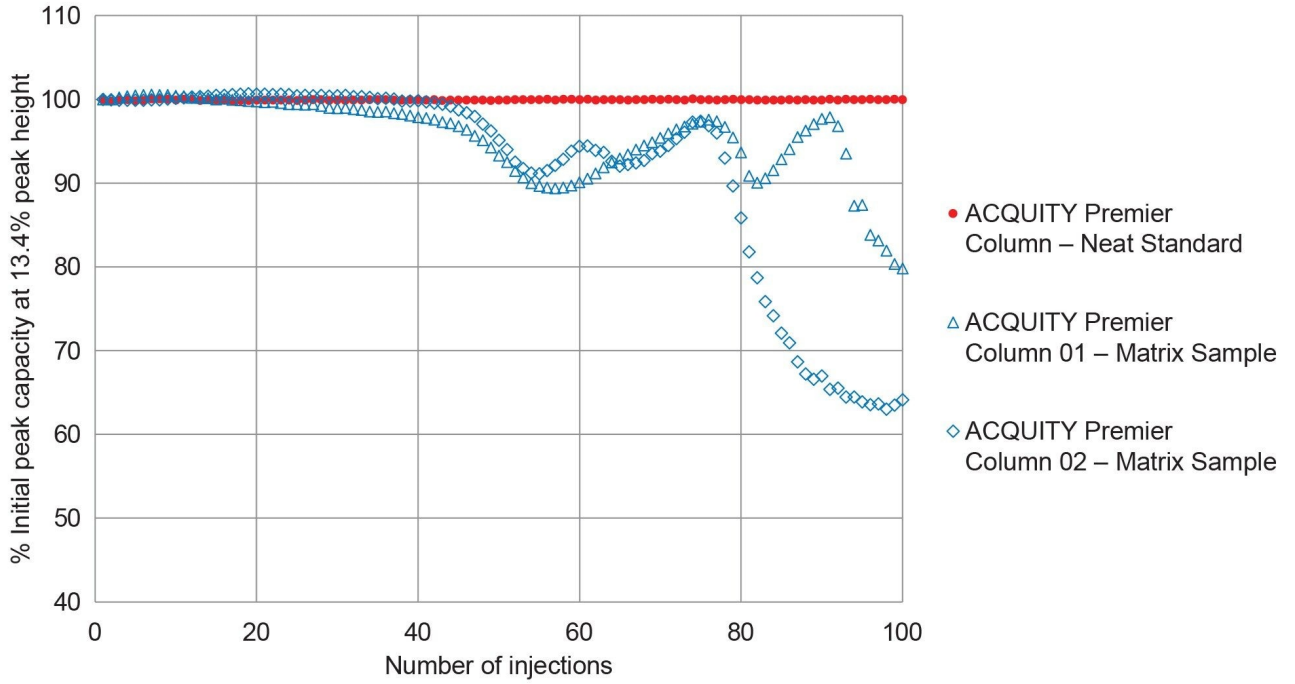

Figure 2. Plot of % initial peak capacity for three columns, two experiencing matrix samples and one experiencing neat standard. Peak capacity measurements calculated using peak with at 13.4% peak height which is also denoted as 4σ.

Both columns which were used to analyze matrix samples start to show performance loss at ~45–50 injections. Once performance loss starts the analytical bed can become compromised leading to worse and worse performance. It was determined therefore that after forty injections the guard cartridge should be changed to reduce contamination of the analytical column. While this seems like a very frequent interval for swapping out a guard cartridge, the frequency depends on the analysis conditions, as well as the sample. Other samples, like plasma, may require a different injection frequency for replacing the guard cartridge. Additionally, the LC method conditions can play a role in column fouling and should be considered when using guards.[3] Using LC conditions which do not adequate clean out excipients between injections allows them to accumulate over time leading to column fouling. The frequency of replacement for guard cartridges should be determine on an assay-by-assay basis depending on the laboratory protocols in place.

Once the frequency of replacement has been determined, the VanGuard FIT column can be put into routine use, monitoring both system pressure and analytical performance to ensure the assay is performing well. Collecting

and monitoring system pressure can be an indicator of when a column will begin to fail, even if the performance is still within an acceptable range. Figure 3 shows the plot of system pressure for the first 130 injections performed on each column. The VanGuard FIT Cartridge was replaced every forty injections as described above.

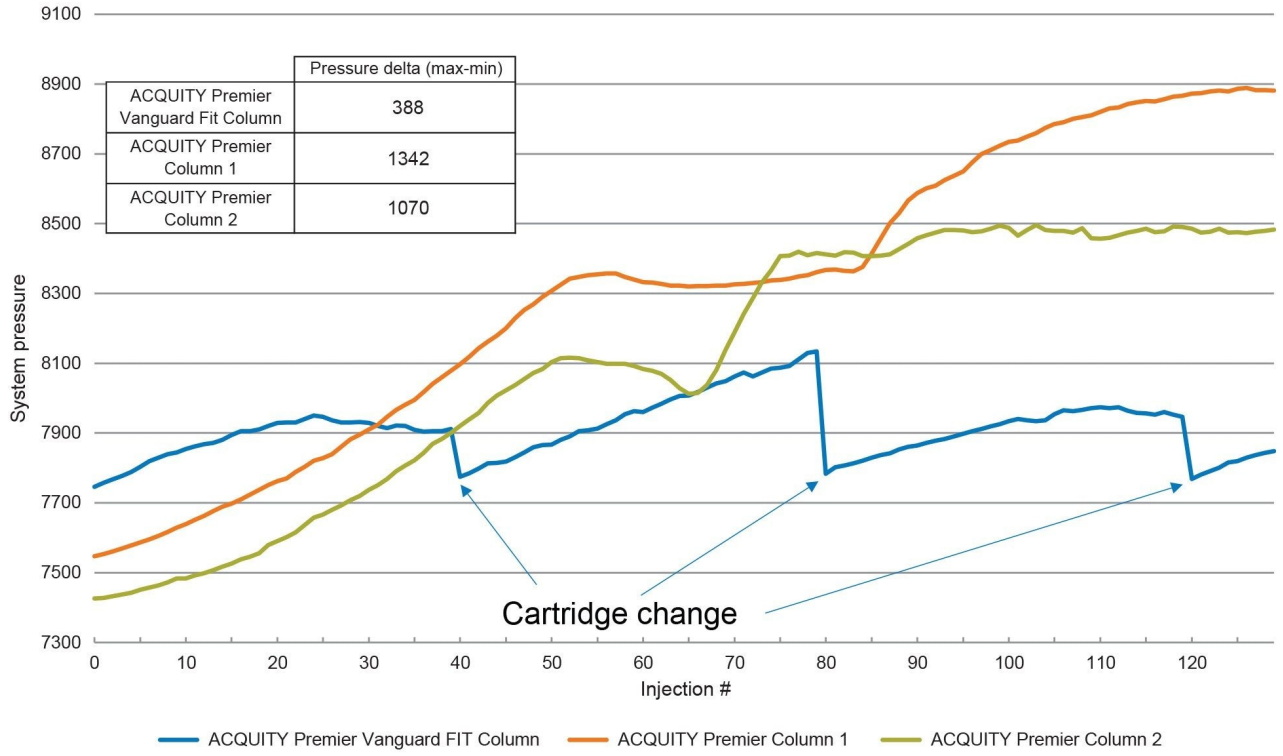

Figure 3. System pressure plots over 130 injections. System pressure recorded at about four minutes during column re-equilibration.

The system pressure increases over the 130 injections for both unguarded columns with the biggest increases occurring early in the testing, between injections one and fifty. The two columns show at least a 1000 psi increase in overall system pressure during this time while the VanGuard FIT Column, with regular changing of the guard cartridge, only showed a pressure delta of <400 psi. In this case, the increased pressure is likely coming from the matrix components which are either clogging the inlet frit or precipitating from the mobile phase due to solubility issues.[3] In either case, these pressure increases are the first indicator that a column is failing or will fail. Unfortunately, system pressure alone can not determine if a column has failed. That determination needs to be made using chromatographic results. Figures 4 and 5 show chromatograms of the

spiked matrix on the two ACQUITY Premier columns (Figure 4) and the ACQUITY Premier VanGuard FIT Column (Figure 5).

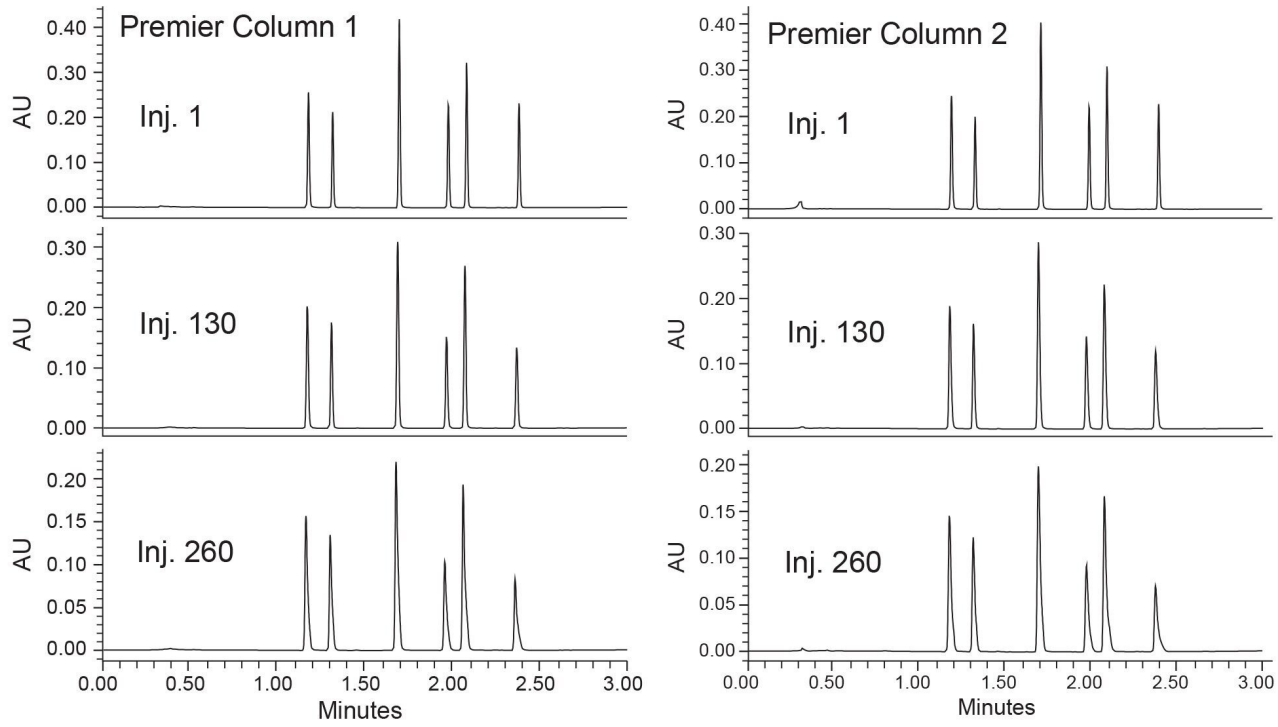

Figure 4. Chromatograms of injections 1, 130, and 260 of spiked matrix on two ACQUITY Premier columns showing performance loss. Elution order of peaks: 2-acetylfuran, acetanilide, acetophenone, butylparaben, propiophenone, valerophenone.

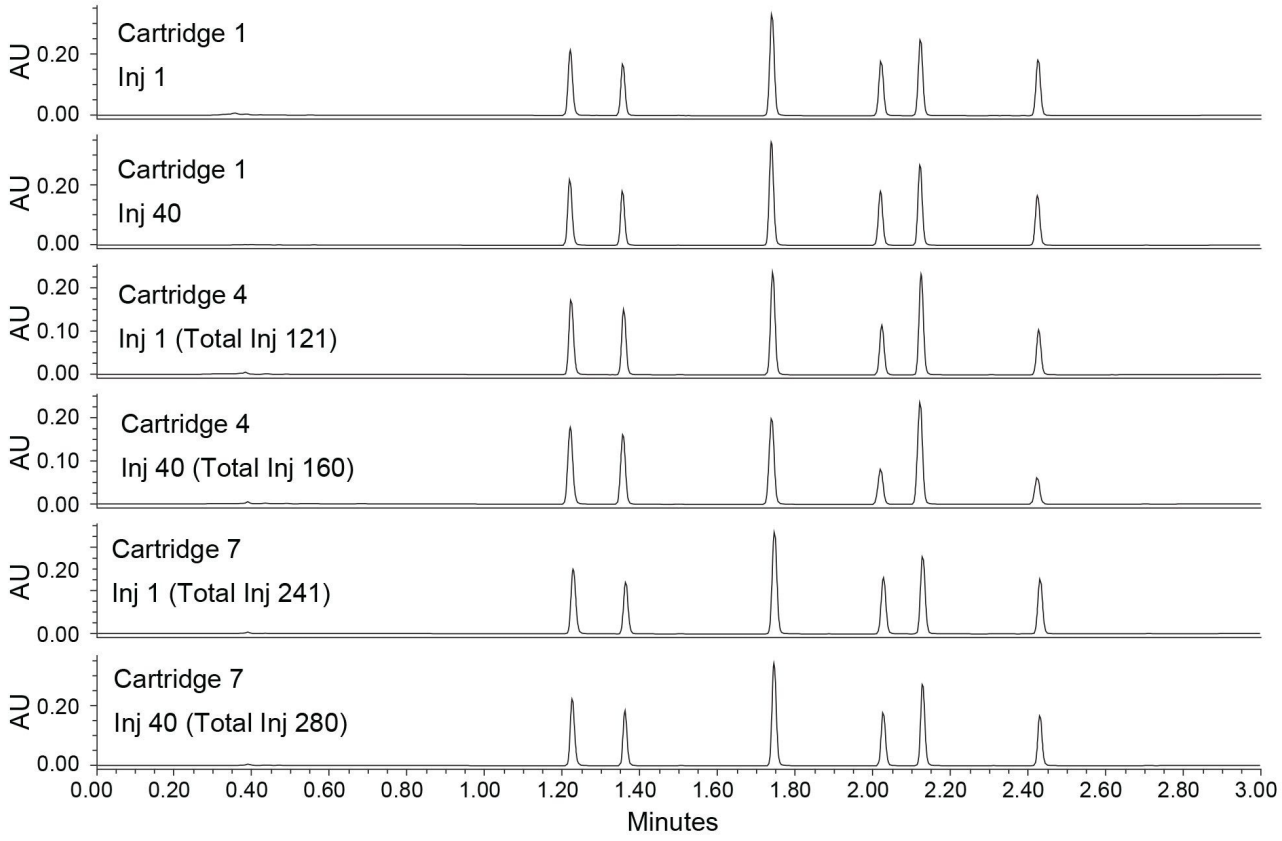

Figure 5. Chromatograms of injections one and forty for each Cartridge 1, 4, and 7 showing early, middle, and late injections on the VanGuard FIT Column. Total number of injections on the column are also shown. Elution order of peaks: 2-acetylfuran, acetanilide, acetophenone, butylparaben, propiophenone, valerophenone.

The unguarded columns show acceptable peak shape at 130 injections compared to injection 1, however when the 260th injection is collected we see some significant differences. Notably, valerophenone and butylparaben are tailing at injection 260 which is indicative of column failure. The VanGuard FIT Column does not have that issue however, as regular changes of the guard cartridges maintain the column's overall performance. Peak shapes do not change between injection one on Cartridge 1 and injection forty on Cartridge 7, a total of 280 injections. Some slight changes in peak height occur but are attributed to sample evaporation and not column performance issues. The continued performance of the VanGuard FIT Column with routine replacement of the cartridge is further proven by plotting the performance of the column in terms of peak capacity over the 200+ injections performed. Figure 6 shows the % of initial peak capacity for each column by averaging ten injections together. By

using the average of ten injections, and plotting % of initial peak capacity instead of peak capacity the data is normalized to draw better comparisons between the columns tested, while also "smoothing" the data in the event of a mis-injection.

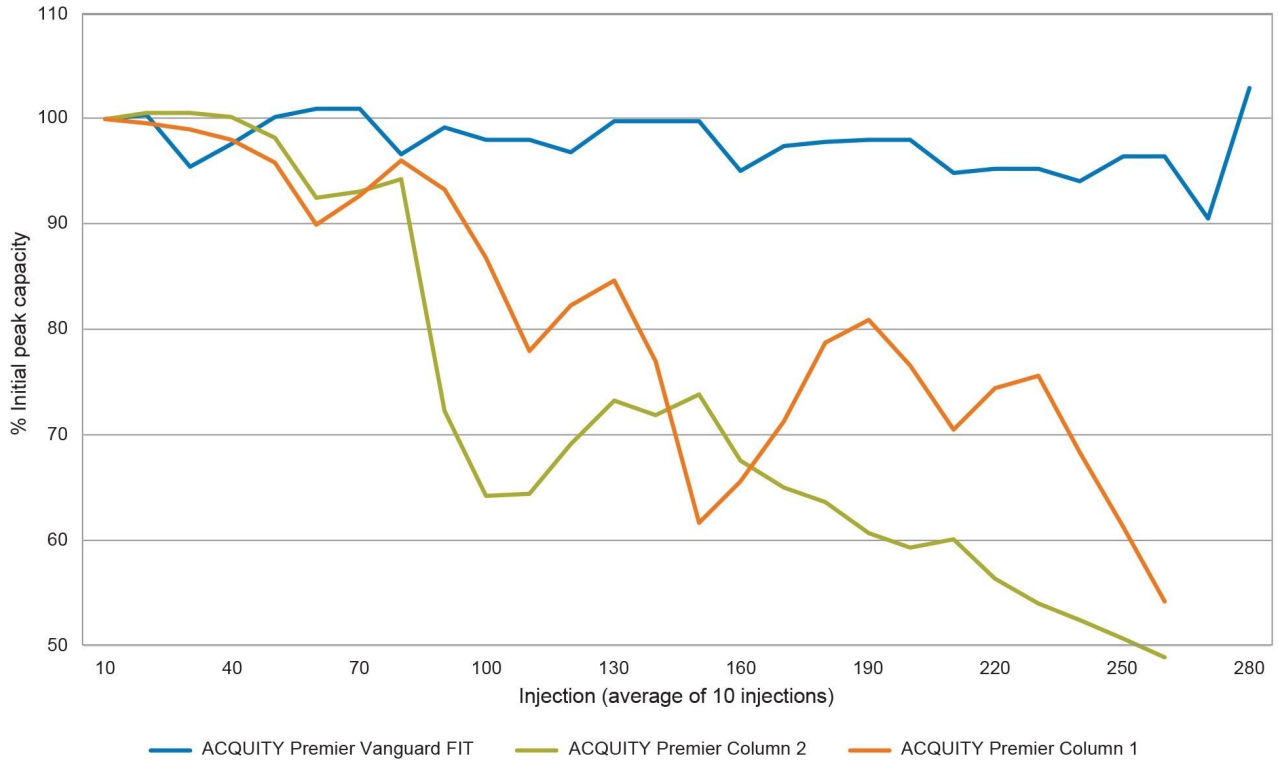

Figure 6. Plot of % initial peak capacity measured at 13.4% peak height for the three columns tested. Average values (n=10) were plotted to reduce the number of data points shown and to minimize the impact of potential mis-injections.

As expected, the VanGuard FIT Column can maintain performance across over 200 injections with less than 10% loss of efficiency seen. When the same number of injections are performed on an unguarded column, the efficiency drops to <60% of initial constituting a major loss in performance. Unguarded columns may offer similar performance at first, and even out to ~40 injections for this sample, both columns start to show major drops in efficiency after that point. Drastic changes occur at ~80 injections however at ~50 injections the performance begins to dip. Using a VanGuard FIT Column in routine testing, especially for samples containing high levels of matrix components which are known to foul columns, can increase column lifetime maintaining the quality of the

data while minimizing the need to replace the analytical column.

Conclusion

To maintain column performance when analyzing complicated samples like plasma or formulated pharmaceutical products sample pre-treatment is often used. However, for some assays, pre-treatment is not viable or allowed. USP monograph methods for instance, are very strict on sample preparation and often only allow for filtration or dilution. The lack of sample pre-treatment in these kinds of assays can introduce significant amounts of excipients or endogenous compounds to the column which could foul it. In instances where sample pre-treatment is not viable, the use of a guard device before the analytical column can greatly increase column lifetime. Guard devices are short, packed beds of stationary phase that can be replaced at regular intervals to prevent fouling of the analytical column. The frequency of replacing the guard device is dependent on the sample being analyzed, and the assay conditions.

The VanGuard FIT Column configuration, available as part of the MaxPeak Premier Column family offers an integrated guard configuration. This hardware allows the use of guards without the additional fluidic connection needed with more conventional guard columns. This ease-of-use design improvement does not impact the ability of the VanGuard FIT cartridges to protect analytical columns from fouling as was shown in this application note. Using VanGuard FIT columns with complicated matrix samples, such as formulated pharmaceuticals or plasma, will help to maintain analytical column performance. Coupled with the MaxPeak High Performance Surface (HPS) technology, VanGuard FIT columns provide significant benefits compared to older guard formats.

References

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3. Berthelette K, Fountain K, Summers M. Improving Method Robustness for Routine Analysis of Pharmaceutical Formulations. Waters Application Note, 720004807, 2013.

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