Application Note

# VanGuard™ FIT (Fully Integrated Technology) Columns: A Practical and Reliable Solution for HPLC Column Protection

Maureen DeLoffi

Waters Corporation

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

## Abstract

In some applications HPLC columns can be subject to particulate material or strongly adsorbing substances becoming trapped in the column, leading to increased column pressure and degraded performance. The most reliable solution to this problem is to use a guard column to prevent particulates and strongly adsorbing substances from reaching the analytical column. A guard column is designed to be replaced periodically, thereby restoring the performance of the separation. Using a guard column addresses the problem of column fouling but has a potential downside of reduced column efficiency and increased peak tailing due to added extra-column dispersion. The VanGuard FIT Column design minimizes extra-column dispersion, enabling users to take advantage of the benefits of guard columns, without sacrificing separation performance.

## Benefits

- Minimal extra-column dispersion for a VanGuard FIT Column versus traditional guard configurations
- Risk of leaks reduced since there are no additional connections to make
- More consistent connections yield more consistent results

## Introduction

The lifetime of an HPLC column depends on many factors, one of which is the extent of contamination due to the build-up of particulates or strongly adsorbed substances. Such contamination can come from several sources, including the mobile phase (e.g. microbes and/or buffer precipitates), the HPLC system, and the sample. Because the column is a highly efficient filter, any particulate material in the flow path or injected onto the column can become trapped. Over time, this may lead to increased column pressure and loss of performance. The most reliable solution to this problem is the use of guard columns or cartridges, which prevent particulates and strongly adsorbed substances from reaching the analytical column.[1] However, the use of guard columns increases the extra-column dispersion, causing a decrease in efficiency and an increase in peak tailing. The extent of this issue depends on the design of the guard column, in particular on how it is connected to the analytical column. In some applications where column efficiency is critical, users may be forced to choose shorter column lifetimes and higher operating costs over the benefits of a guard column.

In this note, we compare the performance of the XP VanGuard cartridge and holder (figure 1a) installed on 2.1 mm XP BEH™ $C_{18}$ 2.5 µm Columns, the ACQUITY™ VanGuard Pre-column (figure 1b) installed on 2.1 mm ACQUITY BEH $C_{18}$ 1.7 µm Columns, and the MaxPeak™ Premier 2.1 mm BEH $C_{18}$ 1.7 µm FIT Column (figure 1c) versus equivalent unguarded columns.

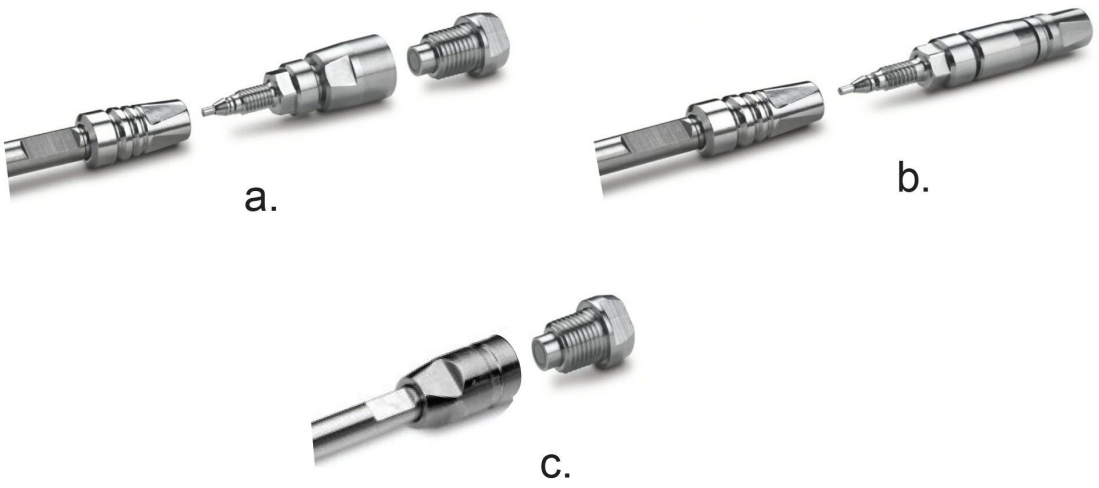


*Figure 1. a) The XP VanGuard cartridge and holder, b) the ACQUITY VanGuard Pre-column, c) the MaxPeak Premier FIT Column.*

## Results and Discussion

### Ease of Use

The first thing a user will notice about the VanGuard FIT Column is its ease of use. The transition tube has been removed from the design, and the cartridge connects directly to the column inlet. Replacement cartridges are easily installed with just a quarter turn of a wrench to properly seal the cartridge into the column inlet. There are no loose ferrules to set improperly and a minimal chance of leaks

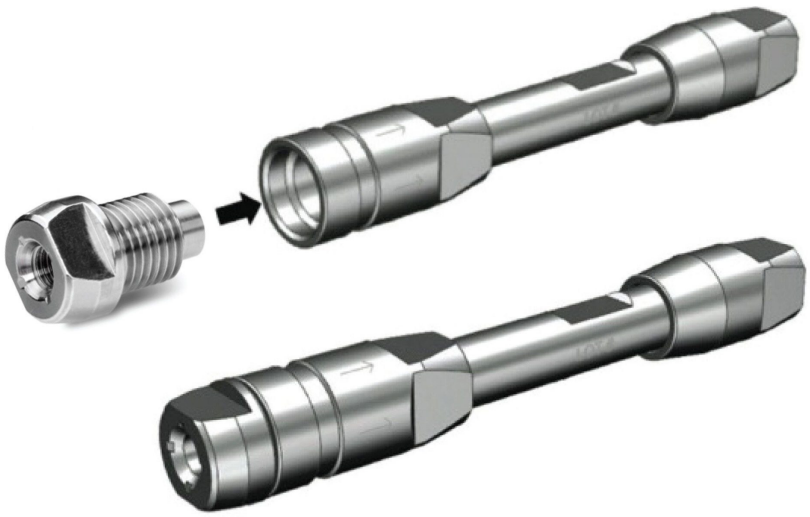

*Figure 2. Assembly of a VanGuard FIT cartridge into a VanGuard FIT Column.*

In contrast, the assembly of a traditional VanGuard cartridge and holder is complex and requires a certain level of manual dexterity for successful installation onto a column. The user must employ two wrenches while simultaneously applying downward pressure to ensure the transition tube is fully seated into the column inlet as the ferrule is set. A void space due to improper protrusion of the transition tube will result in increased extra-column dispersion.

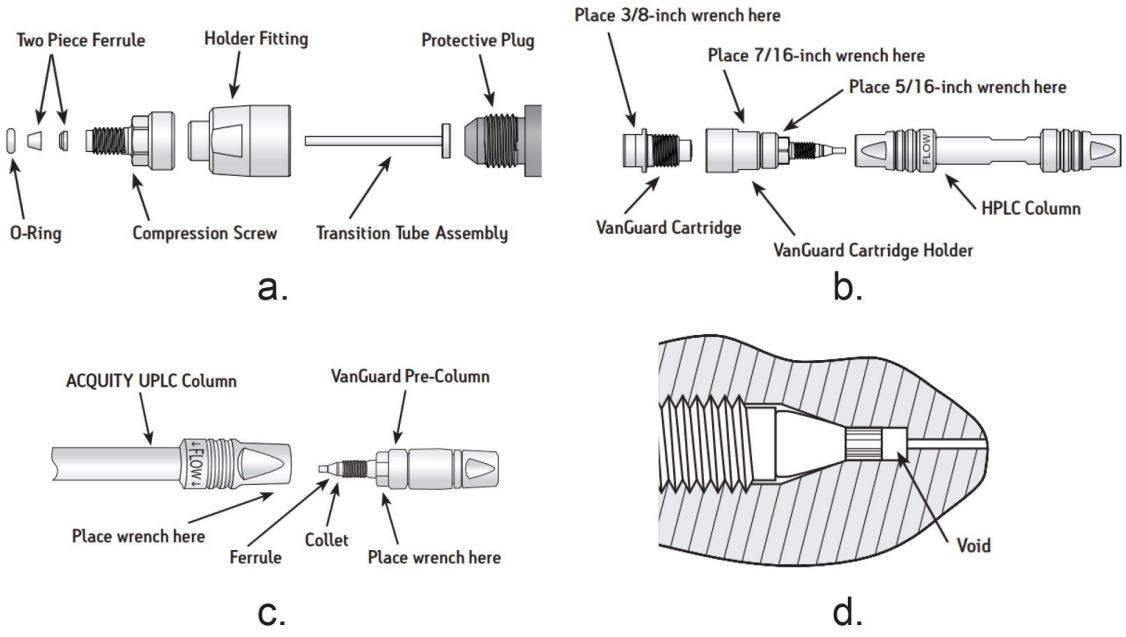


*Figure 3. a) Exploded view of the VanGuard cartridge holder, b) Connection of the VanGuard cartridge, VanGuard cartridge holder, and analytical column, c) Assembly of a VanGuard Pre-column to an ACQUITY UPLC™ Column, d) Void space at the end of the transition tube due to improper VanGuard Pre-column ferrule setting.*

To illustrate how assembly complexity can affect column performance, three people were asked to assemble an XP VanGuard cartridge and holder onto 2.1 x 100 mm XP BEH $C_{18}$ Columns. Table 1 shows the percent effect on the responses of USP column efficiency and USP tailing factor, where the percent effect is calculated as:

$$\frac{\textit{Response with Guard} - \textit{Response without Guard}}{\textit{Response without Guard}} \times 100$$

| Assembler (Avg. 2 columns) | Percent effect USP column efficiency | Percent effect USP tailing factor |
|---|---|---|
| Person A | -7.5 | 5.2 |
| Person B | -17.7 | 11.2 |
| Person C | -6.6 | 11.7 |

*Table 1. Percent effect on USP column efficiency and USP tailing factor for XP VanGuard cartridges and holders connected to 2.1 x 100 mm XP BEH $C_{18}$ Columns by three different people. Variability in the results was noted based on differences in the order of operations during assembly. Columns were tested under isocratic reverse phase conditions using a neutral analyte with a retention factor of ~2.1.*

Not all users referenced the installation instructions[2,3] before assembly. Person A and Person C correctly assembled the holder onto the column and then installed the cartridge. Person B first installed the cartridge into the holder and then installed the assembled unit onto the column. Variations in installation technique can lead to unacceptable column performance differences across users.

## Effect on Analytical Column Performance

The main benefit of the VanGuard FIT guard cartridge is the minimal effect it has on column efficiency. The extra-column dispersion is significantly reduced compared to typical guard columns, as the transition tube has been removed from the design. The cartridge seals directly into the column inlet, resulting in consistent, reliable, and leak-free performance.

To study the effect of the ACQUITY VanGuard Pre-column and the XP VanGuard cartridge with holder, we packed two sets of 18 columns with either 1.7 µm BEH $C_{18}$ material or 2.5 µm BEH $C_{18}$ material. Six columns of each configuration/particle size combination were included in the study.

We first measured the USP column efficiency and USP tailing factor for each column, then installed a VanGuard Pre-column (1.7 µm) or a VanGuard cartridge with holder (2.5 µm) before re-testing to calculate the percent effect. Column efficiency was measured under isocratic reverse phase conditions with a neutral small molecule analyte with a retention factor of ~2.1. Note that in all cases, a brand-new pre-column or cartridge with holder was installed, as opposed to moving the same guard from one column to the next. From the data in Figure 4, we

see that the BEH $C_{18}$ 1.7 µm VanGuard FIT Columns have equivalent efficiency to unguarded columns, while traditional guard configurations yield a ~10% loss in column efficiency.

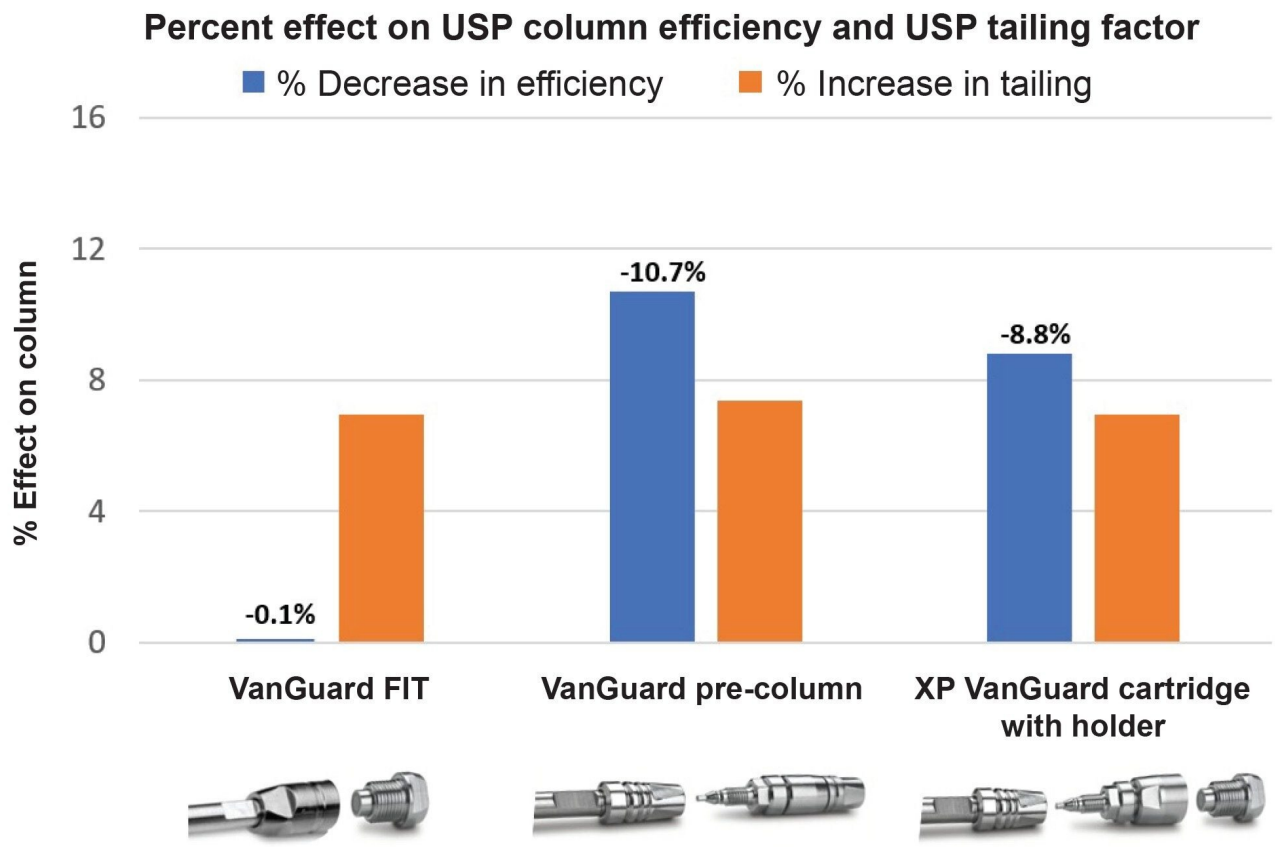


*Figure 4. Average percent effect of the VanGuard FIT vs the VanGuard Pre-column vs the XP VanGuard cartridge with holder on both column efficiency and tailing factor compared to unguarded BEH $C_{18}$ columns in multiple 2.1 mm configurations.*

VanGuard FIT Columns are assembled and tested by Waters™ Operations, allowing direct comparison of the efficiency of a VanGuard FIT Column to an equivalent column without a guard. A review of thousands of data points for BEH/CSH™/HSS sub-2-µm chemistries indicated that on average a VanGuard FIT Column has only 3.2% less efficiency for 2.1 mm columns versus an unguarded column of equivalent dimensions (see Figure 5). This is more than three times less than the average 10.5% effect measured for VanGuard Pre-columns installed on ACQUITY UPLC Columns (n=3 for each chemistry).

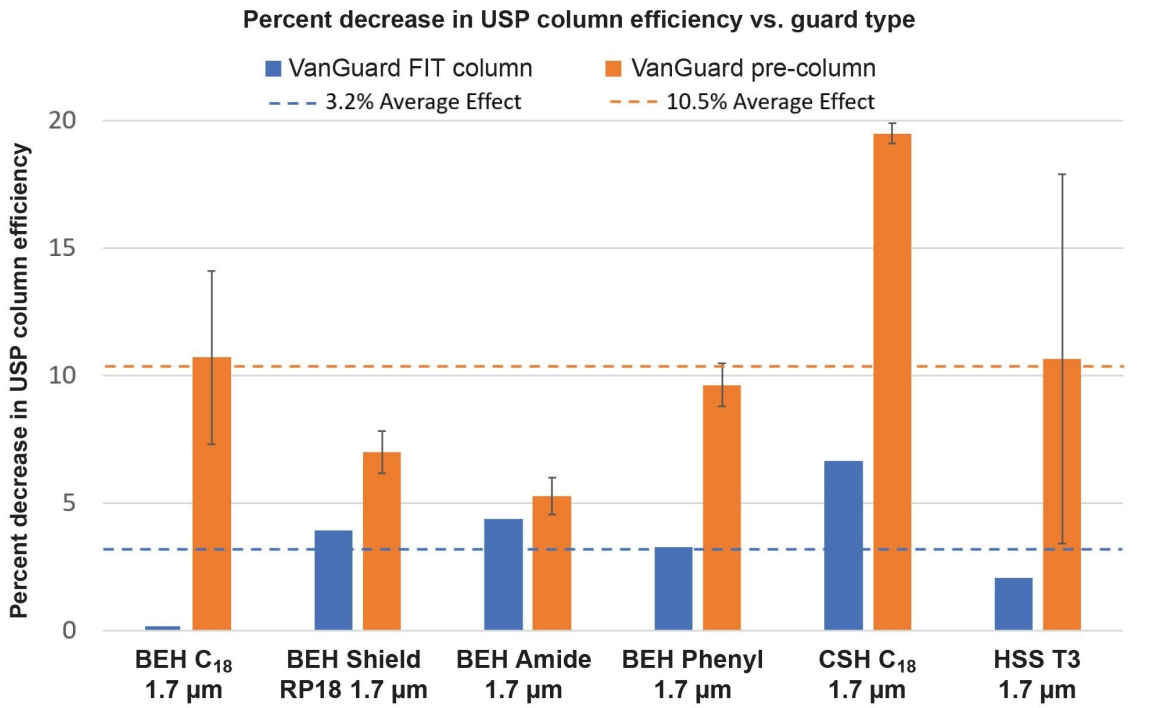


*Figure 5. Percent effect on column efficiency vs guard type. Manufacturing data for FIT columns is compared to equivalent dimension MaxPeak Premier Columns. Experimental data (N=3 for each chemistry) for VanGuard Pre-columns installed on ACQUITY UPLC Columns is shown for comparison. Error bars indicate one standard deviation of the experimental data averages.*

| Guard type | Effect on USP column efficiency | Effect on USP tailing factor | Ease of use |
|---|---|---|---|
| VanGuard FIT | ✓ | ✓ | ✓ |
| VanGuard pre-column | ✓ | ✓ | X |
| VanGuard cartridge with holder | ✓ | ✓ | X |

*Table 2. Summary of effect on USP column efficiency, effect on USP tailing factor and ease of use for three guard types.*

## Conclusion

Because VanGuard FIT Columns are easier to assemble and yield almost no detrimental effect on column efficiency, they are ideally suited for any application that would benefit from the use of a guard column. The VanGuard FIT Column effectively protects the analytical column from degraded performance due to particulate matter being trapped in the bed,[4] but the impact on column performance versus an equivalent unguarded column is significantly reduced. The VanGuard FIT Column also improves the consistency of results between users by eliminating the sources of variability that can occur with traditional guarded columns.

## References

1. Berthelette KD, Alden BA, *et al.* Extending Column Lifetime using VanGuard™ Fully Integrated Technology (FIT) Column Protection. Waters Application Note 720007713. 2022.

2. VanGuard Column Protection Care and Use Manual. 720005317 <https://www.waters.com/webassets/cms/support/docs/720005317en.pdf> .

3. ACQUITY UPLC and ACQUITY Premier BEH Columns Care and Use Manual. 715001371 <https://www.waters.com/webassets/cms/support/docs/715001371.pdf> Rev. K. 2021.

4. Shiner S, DeLano M, ***et al.*** VanGuard FIT: A Breakthrough in Guard Column Performance for Challenging Chromatographic Separations. Waters Application Note 720006500. 2019.

## Featured Products

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

Empower Chromatography Data System <https://www.waters.com/10190669>

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