

Increasing UHPLC Column Lifetime without Sacrificing Separation Quality

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Abstract

Within the last 6 to 8 years there has been a significant movement from using HPLC to using UHPLC separation technology. The most common formats of UHPLC columns are packed with sub-2 μm fully porous media or sub-3 μm core-shell media. The use of this smaller media requires lower porosity retaining frits to be used; thereby making the columns less tolerant to dirty samples. Typically, one uses a guard column or cartridge to increase column lifetime with dirty samples; however, traditional guard systems prove inadequate for several reasons. The first reason is that these columns are usually smaller in inner diameter with 2.1 mm ID columns being the most popular format. Smaller ID columns produce peaks with

smaller volumes, making them less tolerant to extra column dispersion. Traditional cartridges and columns add extra column dispersion, and therefore counteract the performance benefits of UHPLC columns. Another reason is that when using UHPLC columns packed with sub-2 μm particles, the backpressure generated is significantly higher than traditional HPLC separations, causing the traditional columns and cartridges to leak. In this paper, we will show a new generation column guard system that increases UHPLC column lifetime without sacrificing the performance benefits of the UHPLC separation.

Introduction

While analytical HPLC columns have always been considered consumable commodities, they may also represent a significant investment in laboratory operational funds. When dealing with dirty assays, a popular method to protect the investment of the analytical HPLC column has been the use of a guard column. These are very short columns or cartridges (**Figure 1**) placed before the analytical column to trap particulates as well as compounds that exhibit irreversible adsorption to the analytical column. By using guard columns, one would replace a much cheaper consumable; thereby protecting a higher investment of an analytical column. Within the last 5 to 7 years there has been the introduction of UHPLC column technology. Although these columns can consist of different architectures, the use of columns packed with either sub-2 μm totally porous or sub-3 μm superficially porous (core-shell) particles are the most popular. These columns provide

much higher column efficiencies than traditional HPLC columns. With this efficiency comes significantly narrower peaks and smaller peak volumes. **Table 1** shows some typical values for peak volumes for traditional HPLC as well as UHPLC columns. Generally one does not want to add more than 10 % extra column peak dispersion to their chromatographic peaks in order to maintain the peak resolution. The 10 % peak dispersion is not just limited to the dispersion generated by any guard column system that may be employed but also the extra column volume of the system used to perform their separation. Also shown in **table 1** is the allowable total extra column dispersion volume for the various column formats in HPLC and UHPLC. In this paper we will show how designing a guard system with low dispersion of volumes can protect the next generation UHPLC column from dirty samples while maintaining the resolution that has been promised by these high efficiency columns.

Table 1. Typical peak volumes for HPLC and UHPLC columns and the subsequent total allowable extra column dispersion volume

Column Dimension (mm)	Particle Size (μm)	Peak Volume (μL)	Allowable Extra Column Volume (μL)
150 x 4.6	3	312.0	143.5
50 x 4.6	3	180.1	82.9
50 x 2.1	3	41.1	18.9
100 x 4.6	2.6	90.5	41.6
75 x 3.0	2.6	34.6	15.9
50 x 2.1	2.6	15.8	7.3

Table values based upon the following parameters:

HPLC columns: N = 120,000 plates/m (except 2.1 where N = 100,000 plates/m) Lin Vel = 1.9 mm/s UHPLC columns: N = 280,000 plates/m for 4.6, N = 260,000 plates/m for 3.0, N = 200,000 plates/m for 2.1, Lin Vel = 3.5 mm/s

Figure 1. SecurityGuard ULTRA assembly



Figure 2. Backpressure increases as a function of injection of a sample containing a high MW protein sample

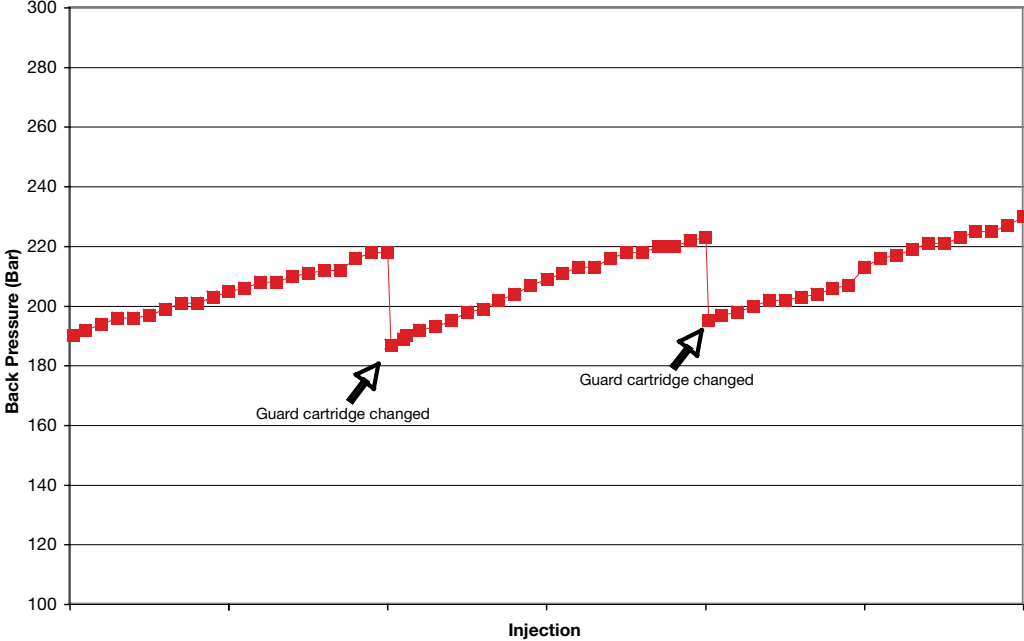
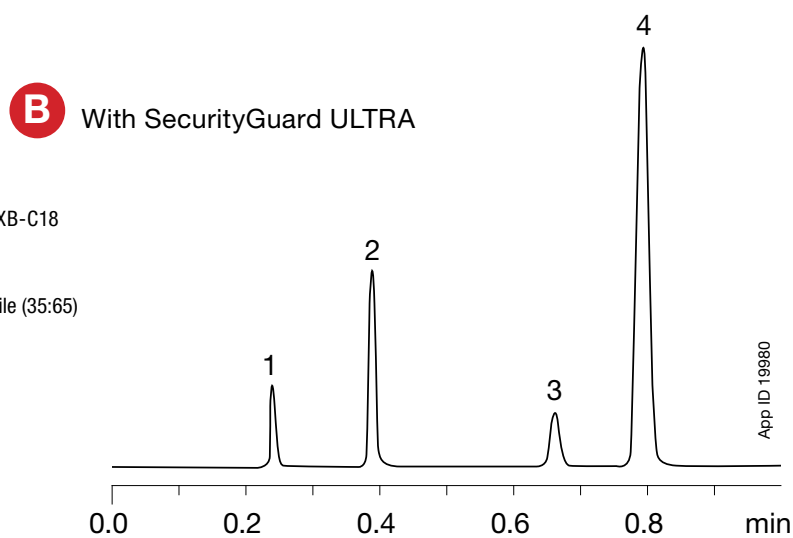
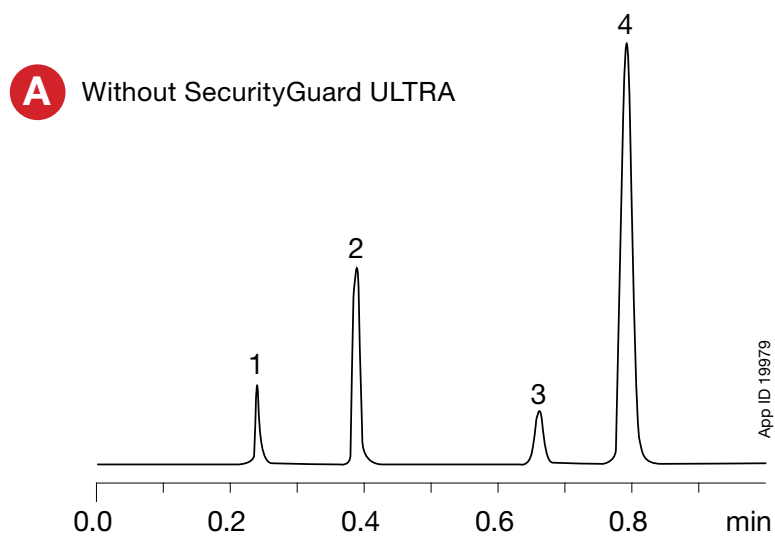


Figure 3. Isocratic test chromatogram comparison using a SecurityGuard™ ULTRA with a Kinetex® 4.6 µm 50 x 4.6 mm column



Column: Kinetex 2.6 µm XB-C18
Dimensions: 50 x 4.6 mm
Part No.: 00B-4496-E0
Mobile Phase: Water / Acetonitrile (35:65)
Flow Rate: 1.85 mL/min
Temperature: Ambient
Backpressure: 155 bar
Sample: 1. Uracil
2. Acetophenone
3. Toluene
4. Naphthalene

Figure 4. Percent of the original column performance tested with 20 different SecurityGuard ULTRA cartridges using a Kinetex 2.6 μm 50 x 4.6 mm column

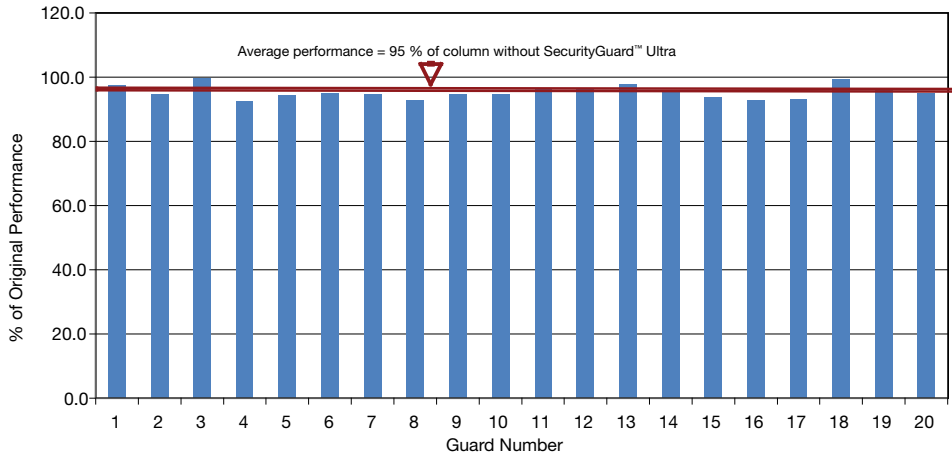
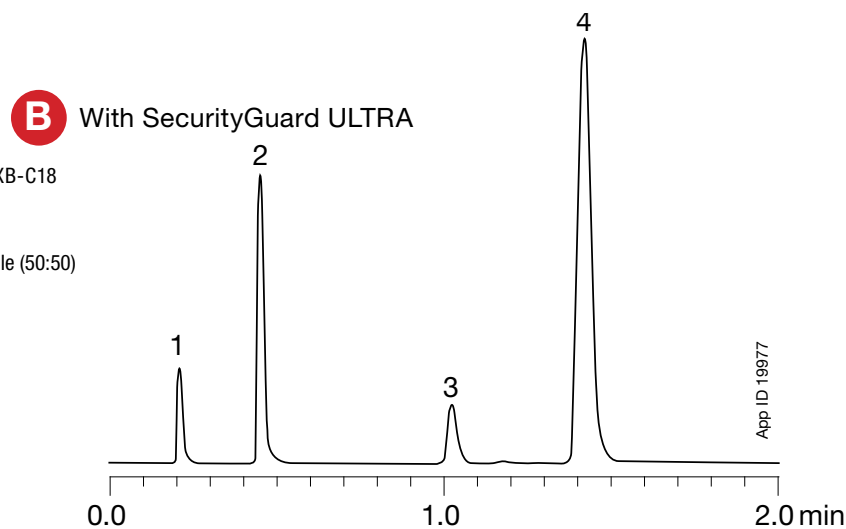
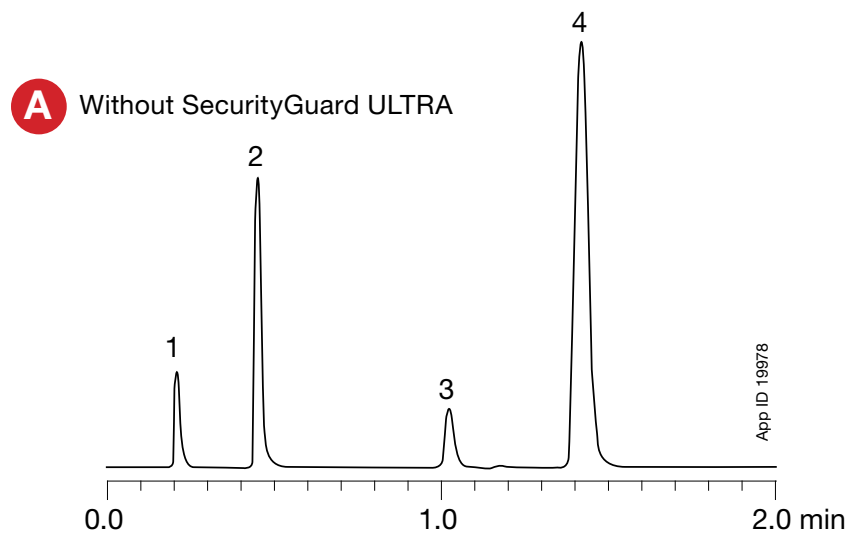
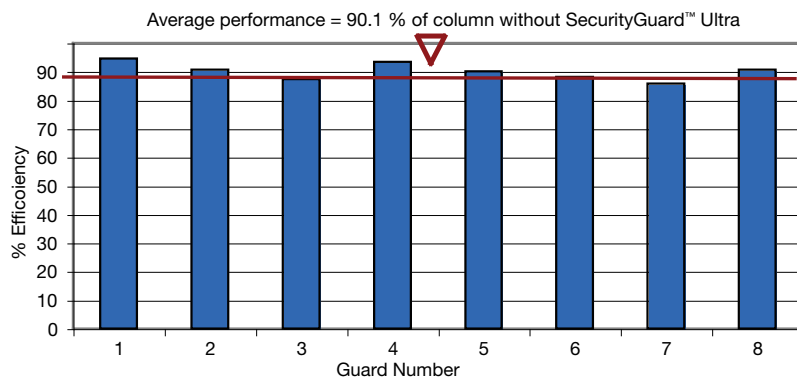


Figure 5. Isocratic test chromatogram comparison using a SecurityGuard ULTRA with a Kinetex 2.6 μ m 50 x 2.1 mm column



Column: Kinetex 2.6 μ m XB-C18
Dimensions: 50 x 2.1 mm
Part No.: 00B-4496-E0
Mobile Phase: Water / Acetonitrile (50:50)
Flow Rate: 0.5 mL/min
Temperature: Ambient
Detection: UV Variable
Backpressure: 230 bar
Sample: 1. Uracil
2. Acetophenone
3. Toluene
4. Naphthalene

Figure 6. Percent of the original column performance when testing 8 different SecurityGuard™ ULTRA cartridges with a Kinetex 2.6 μm 50 x 2.1 mm column



Results and Discussion

Table 1 shows values for peak volume and subsequently the allowable extra column volume for traditional HPLC columns versus UHPLC columns. Looking at one of the most popular dimensions of traditional HPLC columns, 150 x 4.6 mm, the peak volume and allowable extra column volume is 312 and 144 μL , respectively. Typically when going to UHPLC a shorter column is used making the 100 mm length more popular than the 150 mm; between the shorter column, higher efficiency, and increased optimum linear velocity, this decreases the peak volume and allowable dispersion volume to 90 and 41 μL , respectively, or roughly 3.5 times less than traditional HPLC columns. For LC/MS analysis the 50 x 2.1 mm column is a popular dimension. If we look at the peak volume difference between HPLC and UHPLC columns in this dimension we find that the UHPLC columns have 2.6 times less allowable dispersion volume. In both of these examples it is clear that what was traditionally acceptable in terms of extra column band dispersion from guard column systems is no longer appropriate.

Figure 2 shows the backpressure increase as a function of injection number for a high molecular weight protein sample. After a period of time the backpressure increased 15 % versus the initial backpressure. At this point the SecurityGuard™ ULTRA cartridge was changed and the initial backpressure was restored. This process was repeated a second time with similar results. The ability to restore the backpressure by changing the SecurityGuard ULTRA cartridge shows the guard system's ability to successfully protect the column from plugging.

Figure 3 shows the chromatogram from an isocratic test run on a 50 x 4.6 mm column done with and without a SecurityGuard ULTRA. While there was a slight decrease in column performance (5 %), there was no noticeable change in the chromatography.

Figure 4 shows the relative percentage performance versus the original column performance when 20 different SecurityGuard™ ULTRA cartridges were tested with a 50 x 4.6 mm column. The lowest performing cartridge provided 93 % of the original column performance without the guard, whereas the highest performance was 99 % of the column performance without the guard. The average performance was 95 % of the column performance without the SecurityGuard ULTRA, which corresponds to a loss of resolution of only 1 %.

Figure 5 shows a chromatogram of an isocratic test run on a 50 x 2.1 mm column with and without a SecurityGuard ULTRA. While there was a slight decrease in column performance (8 %), it led to no noticeable change in chromatography.

Figure 6 shows the relative percentage performance versus the original column performance when 8 different SecurityGuard Ultra cartridges were tested with a 50 x 2.1 mm column. The lowest performing cartridge gave 86 % of the original column performance without the guard, whereas the highest performance was 95 % of the column performance without the guard. The average was 90.1 % of the column performance without the guard, which corresponds to a loss of resolution of only 1.6 %.

Conclusions

- The SecurityGuard ULTRA system was able to trap particulates from dirty samples.
- By changing the SecurityGuard ULTRA cartridge the original column performance was restored showing the successful column protection.
- The SecurityGuard ULTRA with a 4.6 mm column results in less than 10 % loss in column efficiency (<1.5 % loss in resolution).
- The SecurityGuard ULTRA with a 2.1 mm column lead to a less than 15 % loss in column efficiency (>2 % loss in resolution).

Trademarks

SecurityGuard is a trademark of Phenomenex, Inc. Kinetex is a registered trademark of Phenomenex Inc. in the USA, European Unions and other jurisdictions.

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