

Guard Column Choice: Finding the Balance Between Cost and Analytical Performance

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Introduction

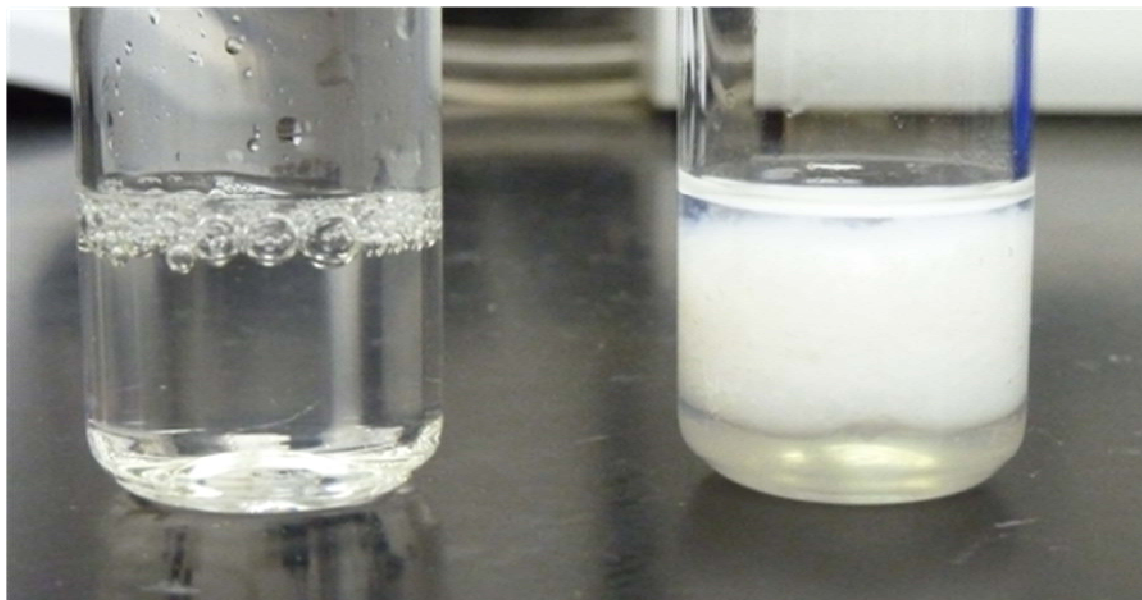
Guard columns can greatly improve the life of an analytical column, especially when analyzing 'dilute and shoot' biological samples. Guard columns act to protect the analytical column from particulates as well as from compounds that may irreversibly adsorb to the HPLC column packing media. Without the use of a guard column, matrix components may collect on the head of the analytical column over time, reducing the performance of the column. In-line frits also play a large part in removing particulates from a sample. In some cases, the use of a low-cost frit alone can improve column lifetime.

Experimental Design

The goal of this experiment was to determine the effect of guard column implementation and column particle size choice on column lifetime under day-to-day analytical conditions. Although the experiment is relatively straightforward, the design of the experiment itself was not trivial. In order to accurately determine the effect of guard columns and frits on analytical results, a method had to be developed to reproducibly cause column failure in a relatively short period of time.

Experimentation was performed with precipitated whole blood and hydrolyzed urine, but it was determined that unprecipitated plasma worked the best to reliably cause column failure. The main reason for this was because while unprecipitated plasma is completely soluble in the starting HPLC mobile phase (95% H₂O), many of the proteins are insoluble when organic concentrations are increased. Figure 1 shows unprecipitated plasma matrix in the starting and ending mobile phases used in this experiment.

Figure 1: Plasma Matrix in Starting and Ending mobile phase



Note that in starting mobile phase (95% H₂O + 0.1% Formic Acid), plasma is soluble, but plasma proteins precipitate out in ending mobile phase (95% MeOH + 0.1% Formic Acid)

In order to monitor performance of the analytical column over its lifetime, a test mix was chosen that included several drug compounds across multiple drug classes. In this way, chromatographic performance could be monitored in the context of active and inactive compounds, early and late eluting compounds, as well as physical parameters, such as column backpressure, which was monitored throughout the experiment.

The experiment was set up to test three conditions: analytical column particle size, effect of an additional 2 μ m frit on a 5 μ m analytical column, and the effect of a guard system on a 5 μ m analytical column. Each of the three conditions were tested identically with unprecipitated plasma, and column performance was monitored using the check mix and conditions detailed in Figure 2. A new analytical column was used for each experimental condition, and column performance was tested prior to any plasma injections. Then, sets of 100 plasma injections were performed interspersed with injections of test mix to total 500 injections, with a test mix injection every 100 injections. Details of plasma preparation and gradient conditions for plasma are given in Figure 2, as well as a representative chromatogram showing an injection of test mix on a new 5 μ m analytical column.

Figure 2: Analytical Conditions for Lifetime Testing and Representative Test Mix Chromatogram

Plasma Injection Conditions:

Mobile Phase A: H₂O + 0.1% Formic Acid

Mobile Phase B: MeOH + 0.1% Formic Acid

Oven Temp: 25°C

Autosampler Temp: Ambient

Flow: 0.5mL/min

Gradient:

Time	%B
0.00	10
0.50	10
0.90	95
1.40	95
1.41	10
1.45	stop

Test Mix Conditions:

Mobile Phase A: H₂O + 0.1% Formic Acid

Mobile Phase B: MeOH + 0.1% Formic Acid

Oven Temp: 25°C

Autosampler Temp: Ambient

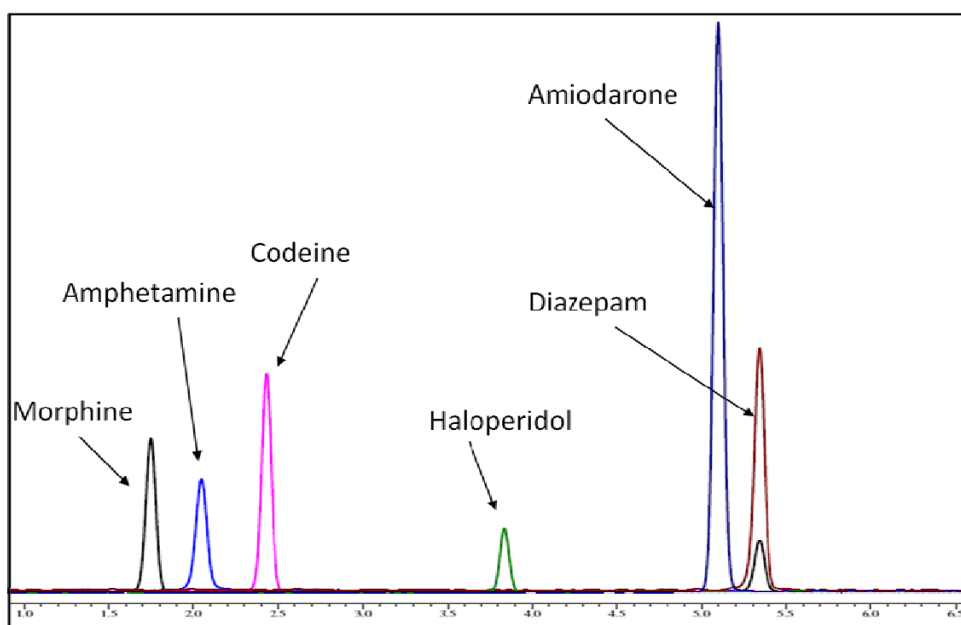
Flow: 0.5mL/min

Gradient:

Time	%B
0.00	5
5.00	95
6.00	95
6.10	5
7.00	stop

Sample Preparation:

Prior to injection, beagle plasma (unfiltered, K2 EDTA preserved) was diluted 1:20 in starting mobile phase for plasma injection conditions. No filtration or centrifugation was performed. For test mix injections, 250µL of Restek cat# 36340 (Forensic Drug Screen Mix) was diluted with 750µL of H₂O + 0.1% Formic Acid.



Results and Discussion: Guard Column Testing

At the conclusion of guard column testing, the following observations were made:

- The use of an additional frit at the head of the column (installed in a Trident Direct frit holder) significantly reduced column backpressure rises due to clogging (Figure 3)
- Guard columns were equally effective at reducing analytical column clogging (Figure 3)
- For some active compounds, especially late-eluting compounds, column lifetime was slightly improved with the inclusion of a guard column (Figure 4)
- For inactive compounds, guard columns did not significantly improve column lifetime (Figure 5)
- For active, early eluting compounds, guard column did not improve analytical column lifetime (Figure 5)

Figure 3: Backpressure Over Column Lifetime

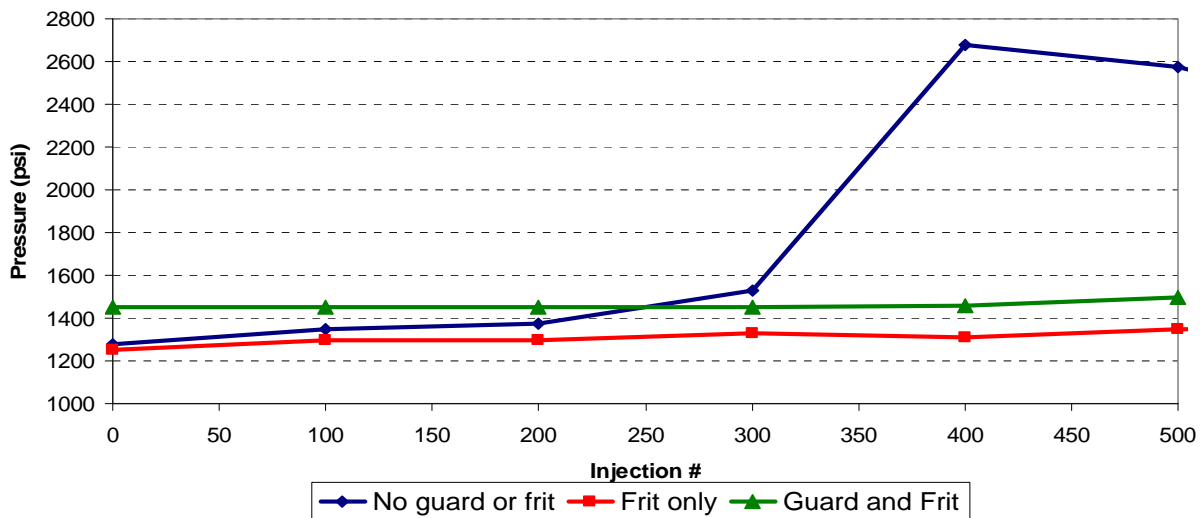
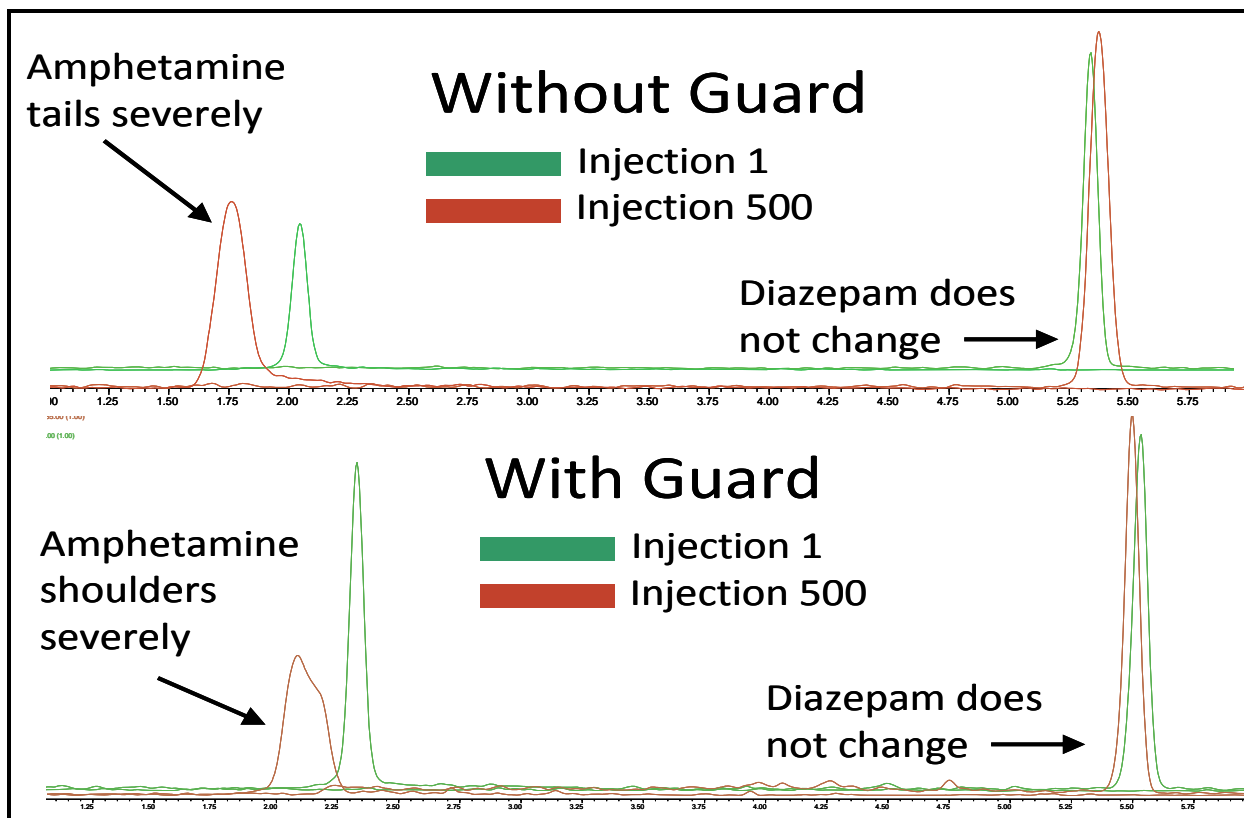


Figure 4: Amiodarone Peak Width Over Column Lifetime



Figure 5: Chromatography of Active and Inactive Compounds



Note in Figure 5 that for both the column with the guard and without the guard, amphetamine shows a marked decrease in chromatographic performance, while diazepam peak shape remains consistent over the life of the column.

Because this testing used a particularly harsh matrix, the effectiveness of the guard column was most probably reduced as compared to more conservative sample matrix preparation methods. Future work will evaluate guard column performance with milder matrices, such as precipitated blood and plasma.

Although the guard testing proved relatively inconclusive, the testing of different particle size columns revealed a large difference in performance between columns of differing particle sizes with biological matrices.

Results and Discussion: Particle Size Testing

At the conclusion of testing columns of different particle sizes and types, the following observations were made. See Figures 6 – 8 for chromatographic data:

- For the conventional (i.e. non-pellicular) particles tested, the main mode of failure was due to loss in retention/selectivity rather than peak shape issues
- The 5 μ m Ultra Biphenyl column showed drastically reduced changes in selectivity and retention as well as the most consistent performance over its lifetime

- All columns exhibited selectivity and retention changes most pronounced after initial exposure to the sample matrix
- Because retention and selectivity remained acceptable throughout the experiment, a 5 μ m, conventional particle column could have a longer lifetime than columns with smaller particle sizes or less initial retention

Figure 6: Chromatographic Performance Over 500 Injections on 5 μ m Particle Column

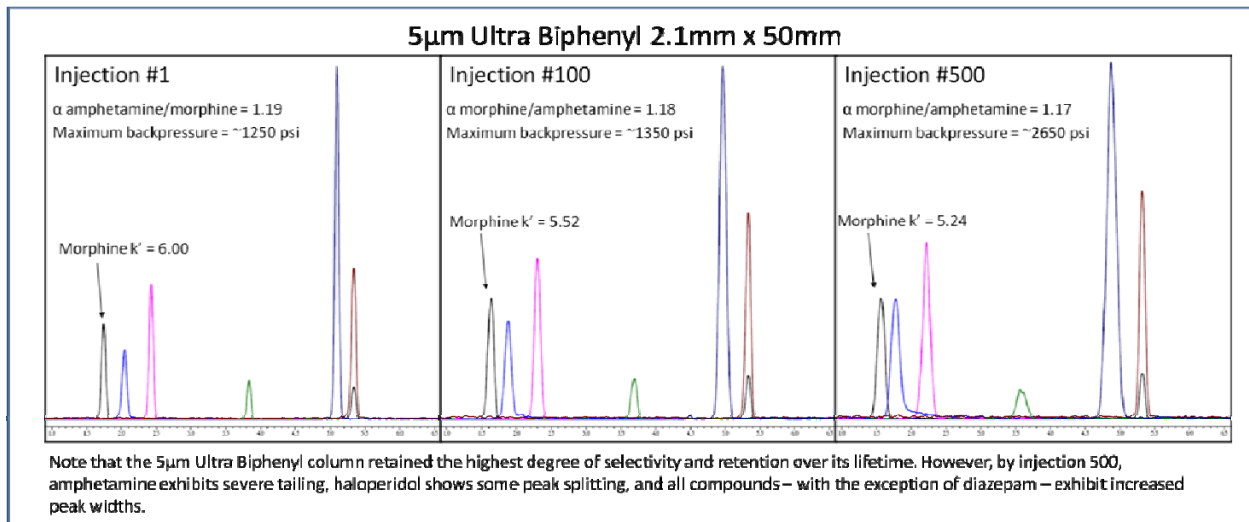
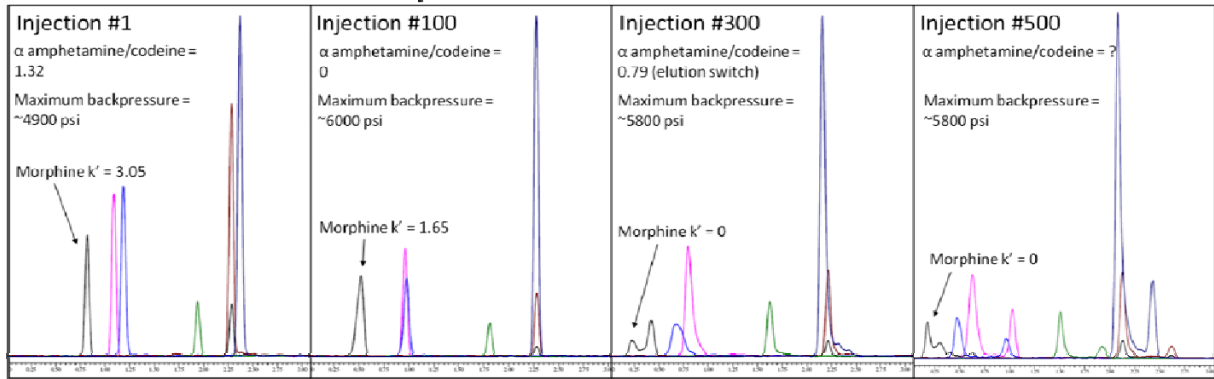


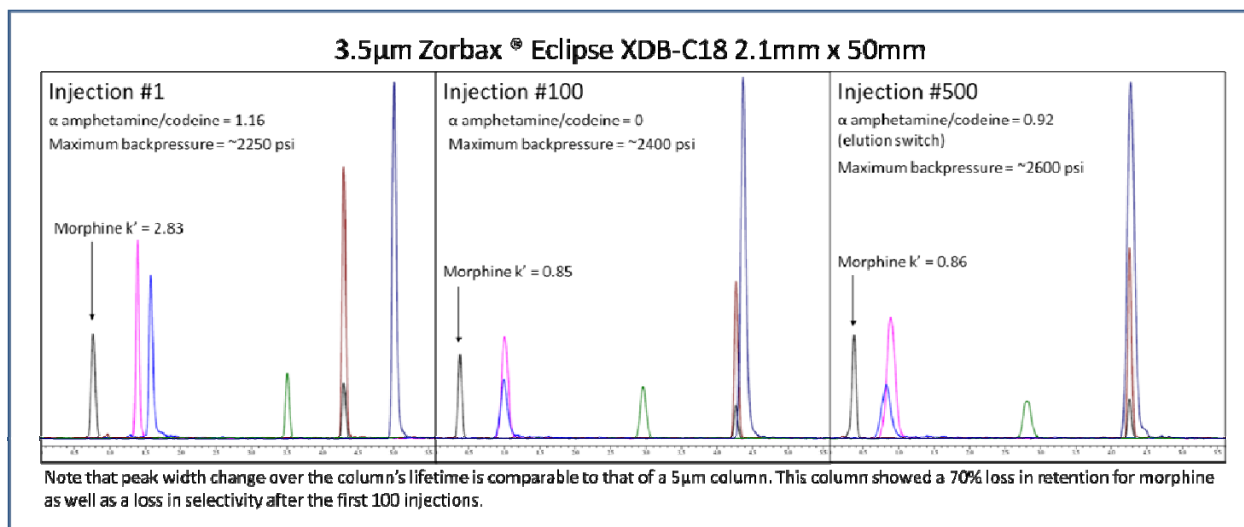
Figure 7: Chromatographic Performance Over 500 Injections on Superficially-Porous Particle Column

2.6 μ m Kinetex[®] C18 2.1mm x 50mm



Note that the 2.6 μ m Kinetex C18 column exhibited a 45% loss in retention for morphine, as well as a loss in selectivity after the first 100 injections. By Injection 300, some peaks are beginning to split, and at Injection 500, all peaks are showing very severe splitting. Alpha measurements for each column were measured between the closest-eluting peaks in the initial analysis.

Figure 8: Chromatographic Performance Over 500 Injections on 3.5 μ m Particle Column



Conclusion

The data presented here shows that although in many cases the implementation of a guard column can be beneficial, the decision to use a guard column should be made based on the nature of both the analytes and the sample matrix for a given method. In some cases, an in-line frit may be all that is required to protect the analytical column. The particle size testing performed during this study shows that columns packed with 5 μ m particles are more robust when subjected to harsh analytical conditions.

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