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Application Note

Improved Chromatographic Performance with an ACQUITY Premier Peptide C_{18} Column Versus a Titanium-Lined C_{18} Column Technology

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

Abstract

Peptides with acidic residues, like phosphorylated post-translational modifications, can often be difficult to recover in liquid chromatography (LC) due to analyte loss via adsorption on electron-deficient metal surfaces, such as stainless steel. Alternative column hardware made of titanium or metal alloys have recently been employed for these applications due to improved corrosion resistance. However, these materials can still contribute to metal-ion mediated sample loss and can make it difficult to recover lower abundant species or peptides with acidic residues and modifications.

With the latest available technology, namely ACQUITY Premier Peptide C_{18} Columns featuring MaxPeak High Performance Surfaces, it is now possible to more easily study metal-sensitive peptide analytes. The MaxPeak High Performance Surfaces provides an effective barrier towards mitigating metal-analyte interactions and any

related loss of sample due to adsorption. In this application brief, we have compared the Waters ACQUITY Premier Peptide CSH C_{18}, 1.7 µm Column to a commercially available titanium-lined positive surface C_{18}, 1.6 µm column.

Herein, we highlight the performance advantage of the ACQUITY Premier Peptide C_{18} Columns in achieving high-quality separations for phosphopeptides and some putative sequence variants that cannot be seen with the titanium lined C_{18} column. For these phosphopeptides, the ACQUITY Premier Peptide C_{18} Column provides improved recovery, better peak shape, and a significant reduction in the need for sample conditioning. In addition, the ACQUITY Premier Column demonstrates greater packed bed stability at high pressures, making it ideal for high throughput applications.

Benefits

- Improved recovery and peak shape versus alternative column hardware
- Higher peak capacity
- Minimal column conditioning required
- Greater packed bed stability
- Higher pressure capability enables higher flow rates for high-throughput applications

Introduction

Adsorption of analytes to metal surfaces has long been a problem in chromatography. Previous mitigation strategies have included passivation of surfaces, use of mobile phase additives, as well as the incorporation of inert hardware materials. While successful to some degree, these strategies have their drawbacks. Passivation of surfaces with either a strong acid, or with sample and/or matrix conditioning are time consuming, require the use of strong acids, and are not long-lived.[1] Mobile phase additives such as chelators can help to prevent analyte-metal adsorption but also have drawbacks including ion suppression, possible solubility issues, and the fact that they must be continually used to remain effective.[2] PEEK columns or PEEK lined steel columns replace the metal surfaces with non-reactive material, but PEEK alone is not tolerant of high-pressure use, and PEEK materials have higher dimensional variability, lower frit permeability, and are incompatible with some solvents.

More recently, columns featuring titanium hardware are now being offered commercially as a more bioinert alternative to conventional stainless-steel columns. Titanium is resistant to corrosion and is inert to some compounds. However, due to its metallic nature, it too can cause analyte adsorption and thus sample loss. Additionally, when used with methanol mobile phases, titanium has been found to leach metal ions.[3]

In this application brief, we compare a commercially available column, lined with titanium and constructed with titanium frits, against an ACQUITY Premier Peptide C_{18} Column, which features MaxPeak High Performance Surfaces (HPS) Technology. MaxPeak HPS is a hybrid organic/inorganic surface technology that has been shown to act as a barrier to the interaction of analytes with metal surfaces. In this work, we demonstrate that the competitor titanium lined C_{18} column suffers from considerably poorer recovery of phosphorylated peptides when compared to the ACQUITY Premier Peptide C_{18} Column. We compared the performance of these two columns both in terms of chromatographic performance using a 4-component phosphopeptide mix, as well as a simulation of the column lifetime capability through means of a packed bed stability test.

Results and Discussion

For this work, we chose a phosphopeptide application to demonstrate the performance differences between an ACQUITY Premier Peptide Column and a commercially available titanium lined column. Phosphopeptides contain anionic phosphate groups that are known to adsorb to the electron-deficient surfaces of metals.[4] Thus, we chose to evaluate the recovery of the Waters MassPREP Phosphopeptide Standard (p/n: 186003285 < https://www.waters.com/waters/partDetail.htm?partNumber=186003285>), a mixture that consists of four synthetic enolase phosphopeptides, one that includes two phosphate moieties (T43pp).

The separation was performed using 0.1% FA modified mobile phase on a titanium-lined positive surface C_{18}, 1.6 µm, 2.1 x 50 mm column or an ACQUITY Premier Peptide CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column. The initial performance of each column was evaluated by means of three repeat injections. Phosphopeptide recovery was determined through UV peak area and observations were also made with respect to peak shape, peak capacity, and column performance after intervals of stress testing, wherein the column was subjected to pressure cycles to simulate accelerated column lifetimes.

Chromatographic Performance

Figure 1 shows the UV chromatograms of the separation on each column while Figure 2 compares the UV peak areas for the T43pp peptide and for the total summed peak area for all four peptides in the MassPREP Phosphopeptide Standard. Peak recoveries for all peaks were higher and more consistent across the initial three injections using the ACQUITY Premier Column. In particular, the doubly phosphorylated peptide (T43pp), which is the most difficult to recover due to its extra acidic phosphate group, was barely recovered upon the first injection using the titanium lined column. The T43pp recovery was only 5% of the recovery resulting from the ACQUITY Premier Column. While the titanium-lined column did improve over time, an indication of pronounced conditioning requirements, the T43pp peptide recovery appeared to stabilize at a level that was still 65% less than that of the ACQUITY Premier Column.

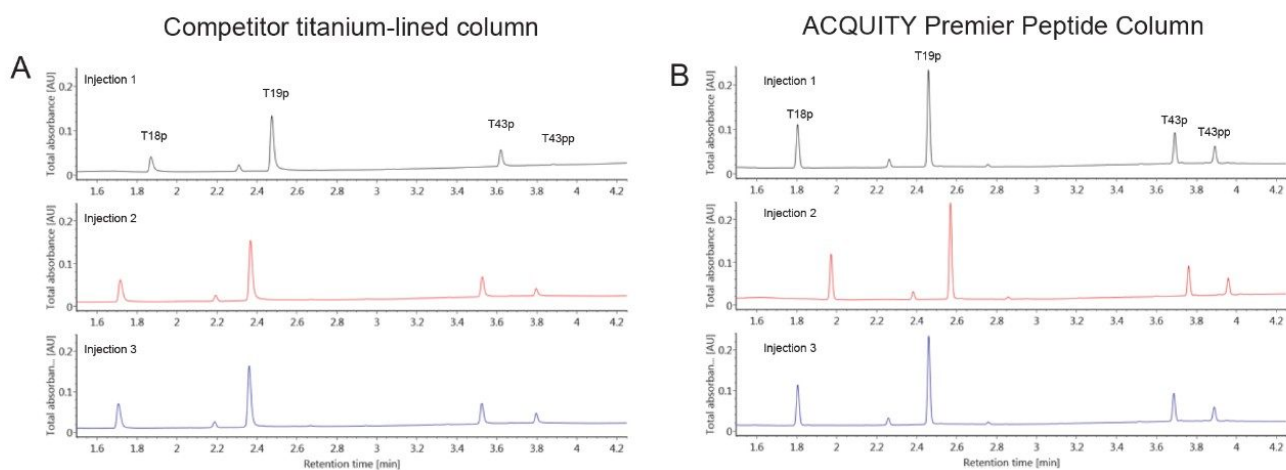

Figure 1. UV chromatograms at 220 nm detection from a separation of the MassPREP Phosphopeptide Standard using 0.1% FA modified mobile phases and (A) a competitor titanium lined, 2.1 x 50 mm column packed with a positively charged C_{18}, 1.6 µm stationary phase or (B) an ACQUITY Premier Peptide CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column. Separations were performed with an ACQUITY UPLC H-Class Bio System, a 0.6 mL/min flow rate, column temperature of 60 °C, gradient of 0.7% to 25% acetonitrile in 5 min, and 200 pmol mass loads.

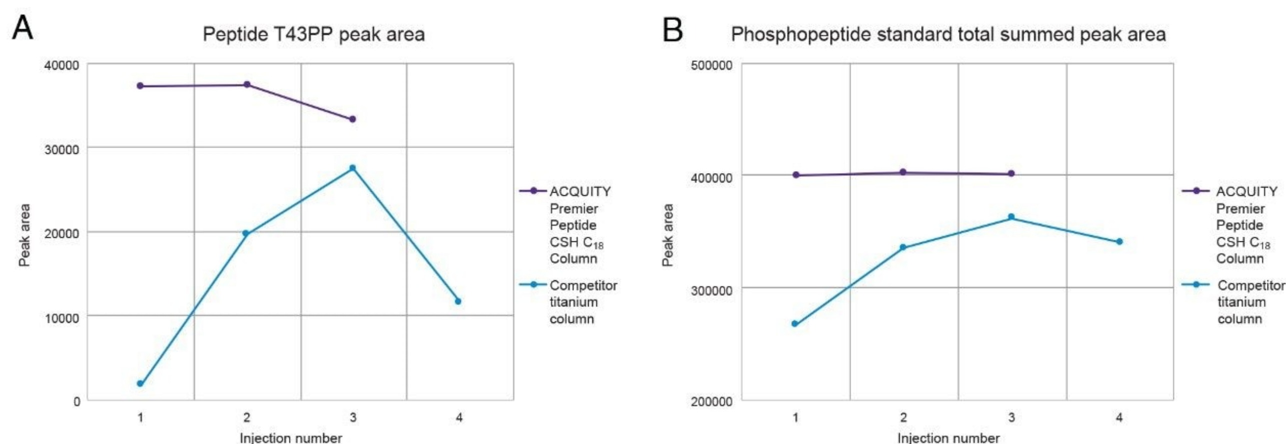

Figure 2. UV peak areas for (A) peptide T43PP and (B) total summed peak area of the MassPREP Phosphopeptide Standard. Analyses were performed with an ACQUITY UPLC H-Class Bio System, 0.1% FA modified mobile phases, and run on a competitor titanium lined, 2.1 x 50 mm column packed with a positively charged C_{18}, 1.6 µm stationary phase or an ACQUITY Premier Peptide CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column. Separation conditions included a flow rate of 0.6 mL/min, column temperature of 60 °C, gradient of 0.7% to 25% acetonitrile in 5 min, 220 nm detection, and 200 pmol mass loads.

When comparing the total summed peak areas of the four peptides using the third injection on each column, we observed a 10% decrease in peak area on the titanium-lined column versus the ACQUITY Premier Column results. After three additional injections on the titanium lined column, the total summed peak area was still 14% lower in comparison to the ACQUITY Premier Column. Overall, the ACQUITY Premier Peptide Column exhibits on the order of 20% higher peak capacity than the titanium lined column. In addition, lower abundant peptide species were observed more readily using the ACQUITY Premier Column. It is likely that these peptide species are sequence variants resulting from the manufacturing of the synthetic peptides in the standard or stability related degradants. That the ACQUITY Premier Column provides more detail across the chromatogram bodes well for its use for a number of different assays, including impurity profiling of therapeutic peptides.

The ACQUITY Premier Peptide Column also exhibits superior peak shape versus the titanium lined C_{18} column. The tailing factor results for each column are depicted in Figure 3. Upon first injection, the average tailing factor was 63% higher using the titanium lined column than on the ACQUITY Premier Peptide Column. After 10 subsequent injections, the average tailing factor for all four peptides was 25% higher. The T43pp peptide, meanwhile, showed a tailing factor that was still 54% higher using the titanium lined column. This suggests that

despite the extra injections, more extreme sample conditioning might be required for the titanium-lined column to approach the lower tailing factors seen with the ACQUITY Premier Peptide Column.

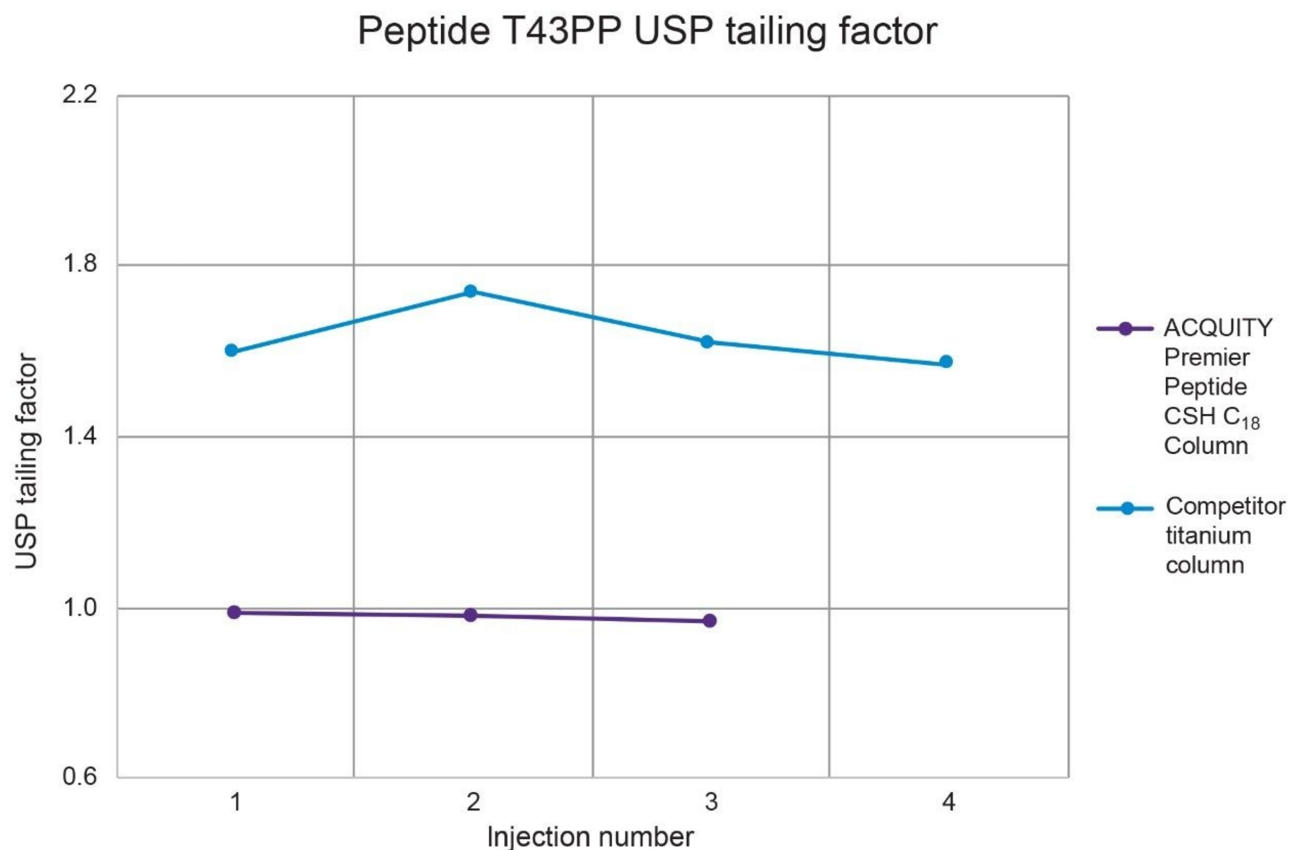

Figure 3. USP tailing factors for peptide T43PP. Analyses were performed with an ACQUITY UPLC H-Class Bio System, 0.1% FA modified mobile phases, and run on a competitor titanium lined, 2.1 x 50 mm column packed with a positively charged C_{18}, 1.6 µm stationary phase or an ACQUITY Premier Peptide CSH C_{18}, 1.7 µm, 2.1 x 50 mm Column. Separation conditions included a flow rate of 0.6 mL/min, column temperature of 60 °C, gradient of 0.7% to 25% acetonitrile in 5 min, 220 nm detection, and 200 pmol mass loads.

Packed Bed Stability

A comparison of packed bed stability was also performed. While a mechanical packed bed stability test may not give a direct indication of column lifetime under normal operating parameters, it can provide an assessment on the maximum performance capabilities of the column.

In this study, each column was subjected to a high flow rate (1.5 mL/min) in order to generate pressures over 11,000 psi on the head of the column. In addition to being subjected to this flow rate, flow was stopped and resumed on each column for many repeat cycles. For the titanium-lined column, we noted a rapid loss in column efficiency and peak tailing after fewer than 200 pressure cycles, as measured with an isocratic separation of acenaphthene. In contrast, the ACQUITY Premier Peptide Column was subjected to over one thousand such pressure cycles with no adverse effects. Figure 4 shows the change in plate efficiency and tailing factor that was observed for acenaphthene throughout this stability test. These results suggest that an ACQUITY Premier Peptide Column is capable of withstanding significantly higher pressures than the commercially available titanium-lined column and is therefore well suited to high throughput applications with high pressures and high flowrates. Despite both columns being rated to an upper tolerance of 15,000 psi, a significant advantage in packed bed stability was found for the ACQUITY Premier Peptide Column versus the titanium-lined column.

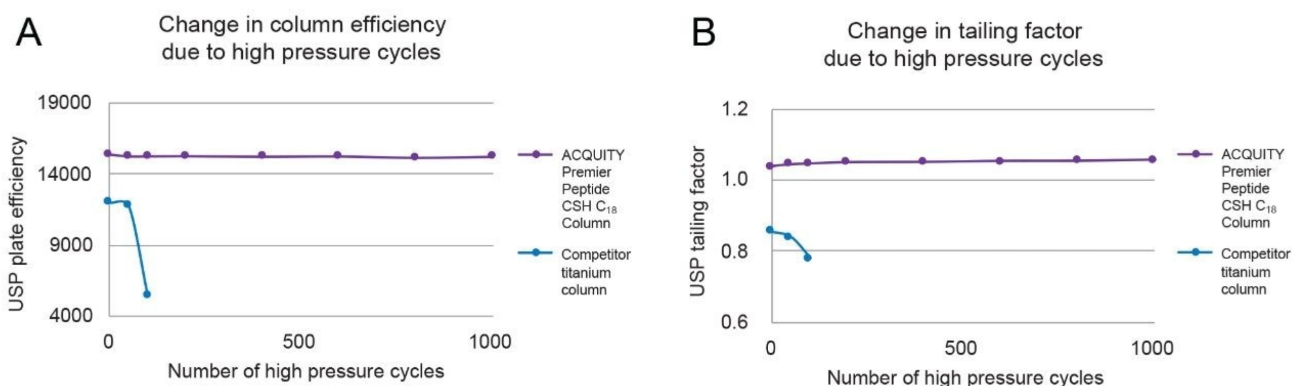

Figure 4. Change in (A) column efficiency and (B) tailing factor on an ACQUITY Premier Peptide Column versus a competitor titanium lined column throughout 1000 high pressure cycles of packed bed stability testing, as measured with an isocratic separation of acenaphthene at a flowrate of 0.35 mL/min, column temperature of 30 °C, and 75% acetonitrile mobile phase.

Conclusion

The ACQUITY Premier Peptide Column with MaxPeak High Performance Surfaces Technology has enabled significant gains in recovery and thus improvements in resolution and peak shape for phosphopeptides and

lower abundant peptide species. The hybrid organic/inorganic technology of MaxPeak HPS offers a barrier to analyte-metal adsorption, granting new improvements in sample recoveries. Gains in the recovery of the doubly phosphorylated T43pp peptide could be seen over multiple injections with the titanium lined column. This suggests a need for sample conditioning. No such performance caveats were observed with the ACQUITY Premier Column, which showed consistent recovery for all four peptides in the study starting with the first injection. A Waters ACQUITY Premier Peptide CSH C_{18} Column does not require extensive conditioning for phosphorylated peptide applications and is ready to use right out of the box.

From these results, we can conclude that ACQUITY Premier Column, with their MaxPeak High Performance Surfaces Technology, have reached a new level of inertness that minimize the adsorption of notoriously challenging peptides, like those containing phosphorylated amino acid residues. Because of this, there is significant promise in ACQUITY Premier Column being used to produce higher fidelity chromatographic data for many different separations, whether they be applied to the study of druggable targets by phosphoproteomics or to detect impurities within synthetic peptide preparations.

References

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ACQUITY UPLC H-Class PLUS Bio System <https://www.waters.com/10166246>

Xevo G2-XS QTof Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>

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