IMPACT OF HPLC INSTRUMENTATION ON NON-SPECIFIC ADSORPTION OF PEPTIDES

Authors: Andrew Steere, Corey Reed, Paula Hong
Waters Corporation, 34 Maple St, Milford, MA. 01757 USA

INTRODUCTION

Peptide mapping is a critical tool in biopharmaceutical analysis for the characterization of protein therapeutics. These separations can be challenging due to the presence of peptides with a wide range of chemical properties, including acidic and phosphorylated peptides. When performing peptide mapping analysis via high-performance liquid chromatography (HPLC), phosphorylated compounds can exhibit non-specific adsorption to the metal surfaces of the instrument.[1] The negatively charged phosphate groups can adsorb to the positively charged metal surfaces due to Lewis acid-base interactions, which then reduces recovery and hinders peak shape for these analytes. Common strategies to mitigate these interactions include the use of mobile phase additives, chemical passivation, or sample priming.[2] However, these solutions can be time consuming and unreliable. For this reason, a variety of technologies have been developed to reduce non-specific adsorption.

In this study, samples containing a wide range of peptides, including those that exhibit non-specific adsorption, are examined across legacy stainless steel HPLC systems and compared to modern biocompatible and bioinert HPLC systems. The analysis was performed using typical reversed phase method conditions and trifluoroacetic acid as an ion-pairing agent in the mobile phase. The performance of the systems was evaluated with comparisons based on chromatographic criteria, including area recovery, repeatability, sensitivity, and peak asymmetry/tailing.

METHODS

There are two methods used to highlight the improved performance of metal sensitive analytes while maintaining the performance of non metal sensitive compounds. The first method uses AMPcP for metal sensitivity and adenosine as a control compound. This sample is made using the Waters Premier AMPcP and Adenosine Standard (part number 186009755).

The second application uses the Waters Phosphopeptide Standard- Enolase Kit (part number 186003285) containing four phosphopeptides with varying metal sensitivities.

For all runs, mobile phases, standards, and samples were prepared by pooling and distributing across all systems to ensure repeatability. This is a critical step as small differences in pH and concentration can lead to large differences in chromatography across the systems. Samples were stored at -20°C and were not exposed to repeated freeze/thaw cycles.

Parameter	Condition
Systems	1) Waters Alliance™ iS Bio HPLC System 2) Legacy HPLC System
Column	XSelect™ Premier HSST3 Column, 2.5 µm, 4.6 x 50 mm, Part Number 186009858
Column Temperature	35°C
Mobile Phases	A) 10 mM ammonium acetate in 99.8/0.2 Water/ Acetonitrile B) Methanol
Flow Rate	1.7 mL/min
Injection Volume	5 µL
Sample Temperature	8°C

Gradient Table

Time	%A	%B
Initial	95.0	5.0
0.30	95.0	5.0
1.10	5.0	95.0
2.60	5.0	95.0
2.80	95.0	5.0
4.50	95.0	5.0

Parameter	Condition
Systems	1) Waters Alliance iS Bio HPLC System 2) Legacy HPLC System 3) Competitor X Bio HPLC System
Column	XSelect Premier Peptide CSH C18 Column, 130Å, 2.5 µm, 4.6 x 100 mm, Part Number 186009908
Column Temperature	60°C
Mobile Phases	A) 0.1% Trifluoroacetic acid in Water B) 0.1% Tri-fluoroacetic acid in Acetonitrile
Flow Rate	0.5 mL/min
Injection Volume	25 µL
Sample Temperature	5°C

Gradient Table

Time	%A	%B
Initial	95.0	5.0
3.0	95.0	5.0
21.0	75.0	25.0
27.0	75.0	25.0
28.0	95.0	5.0
38.0	95.0	5.0

RESULTS AND DISCUSSION

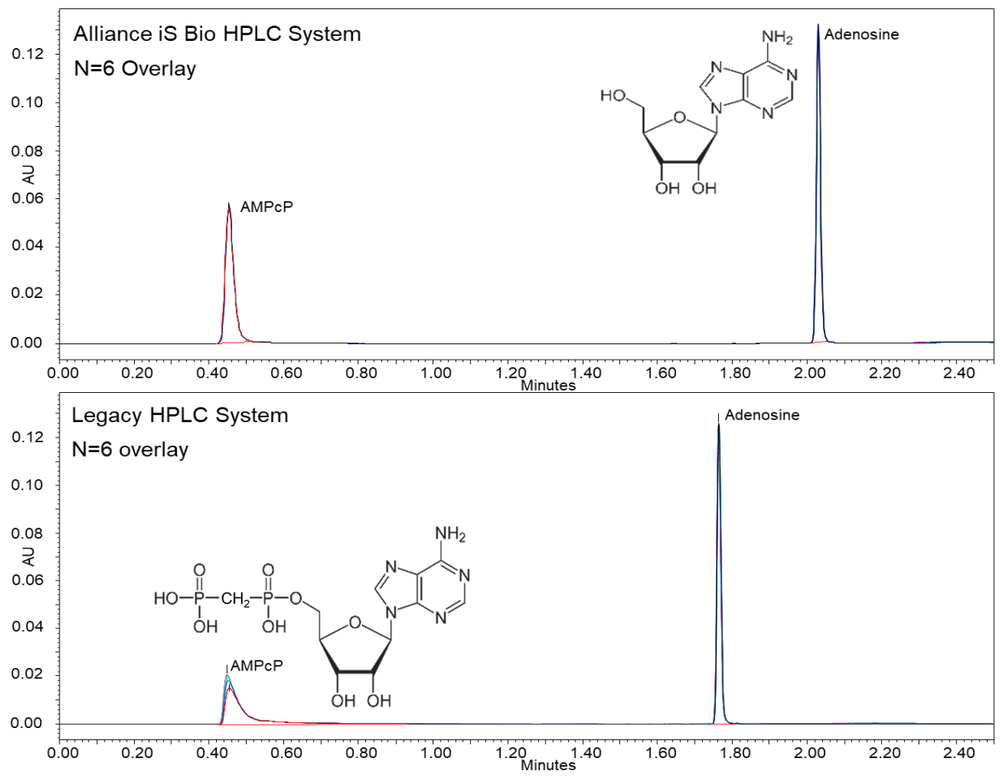


Figure 1. Comparison of n=6 overlay of AMPcP application on Alliance iS Bio HPLC System and Legacy HPLC System and structures of the two compounds

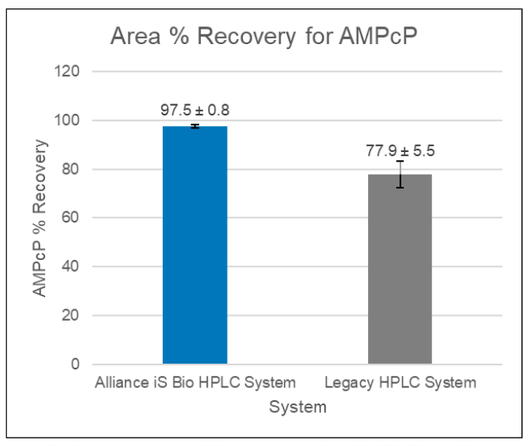


Figure 2. Average area % recovery for AMPcP on the Alliance iS Bio HPLC System and a legacy HPLC system

To the left, the chromatograms show the difference in response and repeatability using the AMPcP and adenosine application on an Alliance iS Bio HPLC System and a legacy HPLC System. The addition of MaxPeak™ High-Performance Surface (HPS) Technology allows the Alliance iS Bio HPLC System to achieve a higher response for AMPcP, a metal sensitive analyte. This is demonstrated by a percent recovery of 97.5% on the Alliance iS Bio System, while the legacy system had a recovery of only 77.9%. The repeatability is also tighter on the Alliance iS Bio System, with a range of only 1.6% compared to an 11% range on the legacy system.

This is also demonstrated visually, where the overlay of the six AMPcP peaks on the Alliance iS Bio System shows very little difference, while on the legacy system there is a drifting response over the course of the six injections. This can be primarily described as system passivation over time, but even once passivated the system still does not perform as strong as the Alliance iS Bio System with MaxPeakHPS Technology. On the other hand, the adenosine peak on both systems is nearly identical. This shows the Alliance is Bio HPLC System is capable of having traditional methods transferred to the system without any worry about changing performance.

After investigating the impact of metal sensitivity using AMPcP, further performance evaluation was explored using a panel of phosphorylated peptides (Figure 3), including three singly phosphorylated peptides and one doubly phosphorylated. Results for this method across three systems are found in Figure 4 below.

The average retention time relative standard deviation is under 0.03% for all peaks on both the Alliance iS Bio System and the Competitor X Bio System, while the legacy system has no peak with a relative standard deviation under 0.08%. This supports the observation of surface passivation when running the AMPcP method above. Due to the inert designs of the bio systems, the chromatography is more repeatable from the first injection.

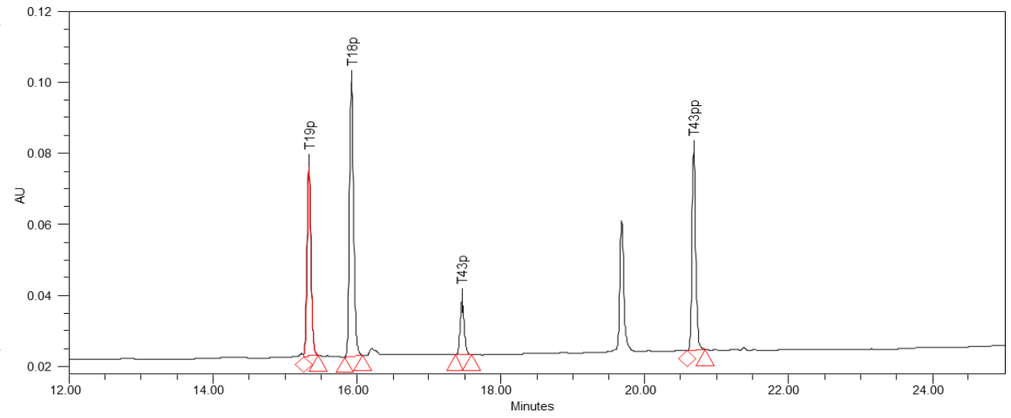
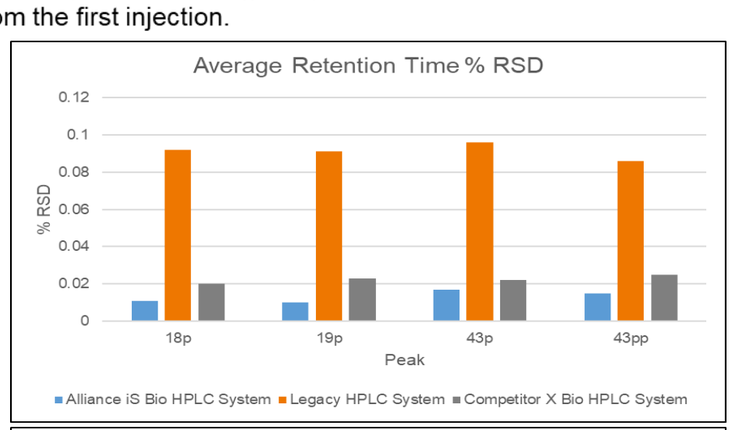
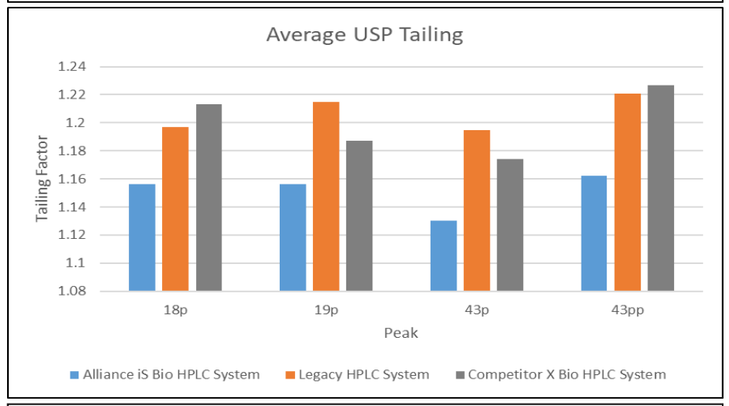


Figure 3. Sample chromatogram of Phosphopeptide application on Alliance iS Bio HPLC System





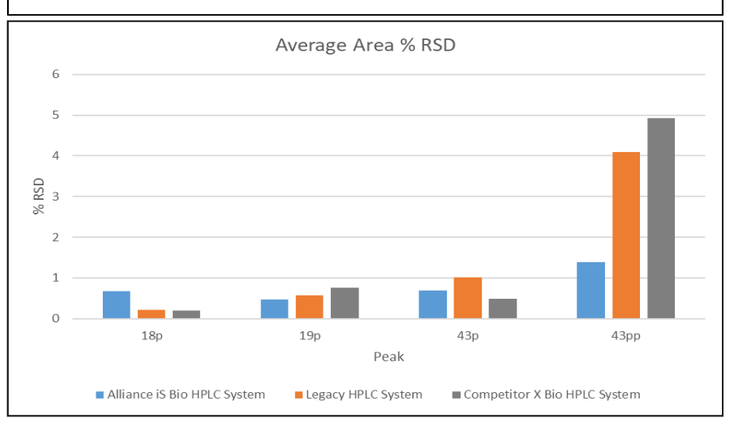


Figure 4. Results across Alliance iS Bio HPLC System, Legacy HPLC System, and Competitor X Bio HPLC System for the phosphopeptide application.

The USP tailing data also supports the inert design of the Alliance iS Bio HPLC System yielding better peak shape and more desirable chromatography. MaxPeak HPS technology within the flow path reduces the surface adsorption of metal sensitive compounds. The tailing factor of all four peaks is the lowest on this system, indicating the most symmetrical peaks. Analytes interact with the metal surfaces found in the flow path of other systems, causing elongated elution and increased tailing.

When looking at system precision, the area percent relative standard deviation remains under 1.5% for all peaks on the Alliance iS Bio HPLC System. This performance is similar to the legacy and competitor systems for the three singly phosphorylated compounds but is significantly better than the other two systems for the doubly phosphorylated 43pp peak. This is noteworthy because the doubly phosphorylated species is more prone to metal interaction than any of the three singly phosphorylated analytes.

CONCLUSIONS

- The Alliance iS Bio HPLC System with MaxPeak High-Performance Surface Technology produces more consistent results with better response compared to a competitor bio HPLC System, particularly for phosphorylated peptides or other metal sensitive compounds
- The Alliance iS Bio HPLC System is capable of transferring methods from legacy systems and maintaining previous results to provide consistency when upgrading products

REFERENCES

1. Reed, C., et al., Improving Peptide Mapping Separations Containing Phosphopeptides Using MaxPeak™ Premier Column Technology on an ACQUITY™ Premier System. Waters Application Note, 2023.
2. Berthelette, K.D., et al., Increased Sensitivity for LC-MS Analysis of Phosphopeptides on an ACQUITY Qda™ Detector Using XSelect Premier Columns. Waters Application Note, 2023.

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS