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Application Note

Bypassing LC System Passivation Requirements Using ACQUITY Premier with MaxPeak HPS Technology for the Recovery of a Phosphorylated Peptide

Brooke M. Koshel, Jennifer Simeone, David Dao, Jennifer M. Nguyen, Susan C. Rzewuski, Matthew A. Lauber, Robert E. Birdsall, Ying Qing Yu

Waters Corporation

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

Abstract

Chemical passivation is a common technique used to enhance chromatographic performance in sensitive applications. Without passivation, analytes that are particularly susceptible to surface adsorption have been shown to suffer from peak tailing and poor recovery or reproducibility when using conventional LC systems and columns. In this study, an LC dedicated to protein and peptide analysis could only recover a phosphorylated peptide after system passivation. Waters ACQUITY Premier with MaxPeak HPS technology was shown to provide an off the shelf solution, bypassing the need for system passivation as required by conventional LC technology.

Benefits

- Increased analyte recovery without the need for system passivation with harsh chemicals
- Improved repeatability for more robust methods compared to conventional LC technology

Introduction

Traditional stainless-steel LC systems and column hardware have the potential to negatively impact chromatographic performance of biomolecules through analyte adsorption to the surface, as well as through free metal contamination when ions are displaced from the flow path.[1] As more modern LC systems have transitioned to corrosion-resistant or biocompatible system components and flow path materials to mitigate this phenomenon, system passivation can still be required for optimal chromatographic performance in applications that are especially sensitive. System passivation works to restore the protective layer that resists corrosion and is often carried out through nitric or phosphoric acid treatments.[1,2] The need for chemical passivation is a more aggressive approach than "conditioning" or "priming" a system or column, which can refer to using repeated injections to block active sites through sample loading or transitioning from storage conditions to running conditions. For many applications, system passivation may never be required, and conditioning or priming are sufficient preparation for achieving the desired performance.

In this study, a peptide mixture containing a phosphorylated peptide is used to highlight performance differences observed between conventional LC technology and ACQUITY Premier with MaxPeak High Performance Surfaces (HPS) technology. Analyte recovery using a conventional LC system and column is only achievable through system passivation using phosphoric acid. MaxPeak HPS technology offers confidence in chromatographic performance through improved recovery, allowing laboratories to run more efficiently by bypassing the need for system passivation.

Results and Discussion

A RPLC gradient using water and acetonitrile with formic acid was used to separate a four-component mixture containing insulin receptor (a doubly phosphorylated peptide having the sequence TRDIpYETDpYYRK), angiotensin I, enolase T37, and bradykinin using a conventional LC system and column. This well-conditioned LC system was a dedicated system for RPLC analysis of proteins and peptides and had performed numerous routine assays meeting various system suitability requirements for retention time repeatability, peak tailing, and analyte recovery. From Figure 1A, only three components of this mixture could be detected. Because insulin receptor is a phosphorylated analyte with a high affinity for metal surfaces, it was completely adsorbed by the wetted flow path. Only after the system was passivated with phosphoric acid could the peptide be recovered (Figure 1B). This same separation was then performed using an ACQUITY Premier Peptide CSH C_{18} Column (130Å 1.7 µm 2.1 x 100

mm) and an ACQUITY Premier System, and all components of the mixture could be recovered without passivation, as was required by the conventional LC technology (Figure 1C). This demonstrates the ability of the ACQUITY Premier technology to provide reliable performance without the need for time consuming system passivation using harsh chemicals.

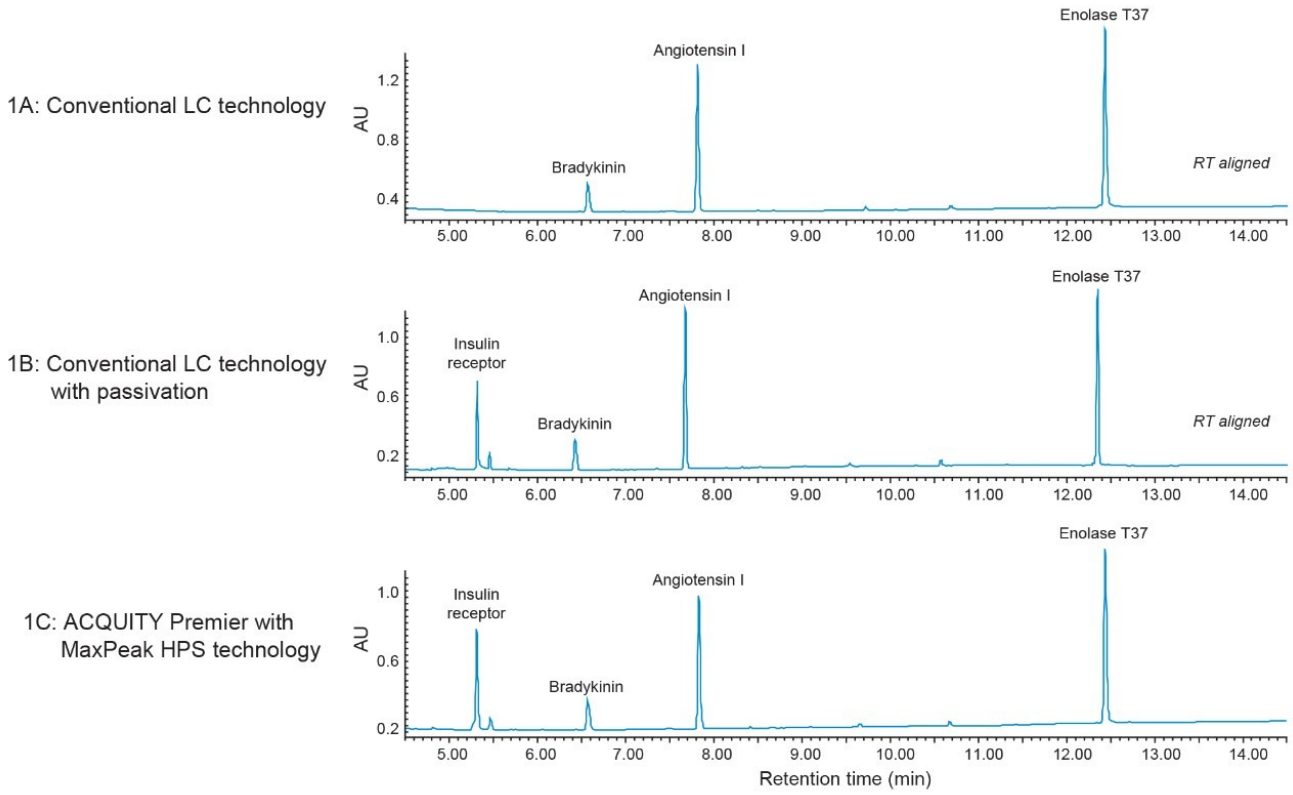

Figure 1: Recovery of insulin receptor, a doubly phosphorylated peptide (TRDIpYETDpYYRK). 1A: Insulin receptor cannot be recovered using "well-seasoned" conventional LC technology. 1B: Adsorption is greatly reduced after system passivation, leading to recovery of insulin receptor. 1C: ACQUITY Premier with MaxPeak HPS technology offers superior chromatographic performance off the shelf, bypassing the need for system passivation required by conventional LC technology.

Method conditions: MPA: 0.1% formic acid in water; MPB: 0.1% formic acid in acetonitrile; gradient conditions: 0.5% to 40% MPB over 12 minutes. A 30% phosphoric acid wash was used for system passivation (Figure 1B only) followed by equilibration at initial gradient conditions.

Furthermore, assay repeatability was also observed to improve using the ACQUITY Premier with MaxPeak HPS technology. Peak area repeatability, which reflects analyte recovery, was compared using conventional technology and MaxPeak HPS technology after system passivation (Figure 2). The %RSD was calculated as 6.22% and 0.48% over 15 injections for conventional technology and MaxPeak HPS technology, respectively. Not only does the MaxPeak HPS technology offer more stable performance over the injection series, but the average

peak area is approximately 1.5 times greater than that reported with conventional technology. Improved analyte recovery and repeatability further demonstrate the advantages of MaxPeak HPS technology for the development of robust methods with enhanced detection of sensitive analytes.

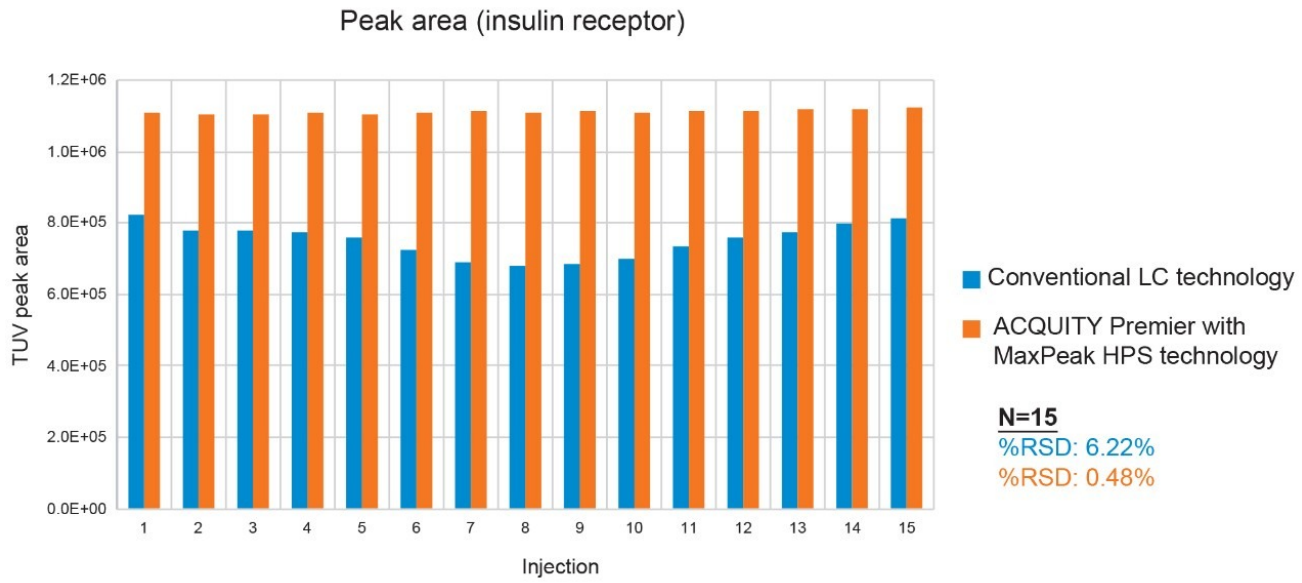

Figure 2. Recovery and peak area repeatability of insulin receptor, a doubly phosphorylated peptide (TRDIpYETDpYYRK). The %RSD across 15 injections was calculated as 6.22% and 0.48% for conventional LC technology and ACQUITY Premier with MaxPeak HPS technology, respectively.

Method conditions: A 30% phosphoric acid wash was used for system passivation followed by equilibration at initial gradient conditions. MPA: 0.1% formic acid in water; MPB: 0.1% formic acid in acetonitrile; gradient conditions: 0.5% to 40% MPB over 12 minutes.

Conclusion

System passivation has become a common practice for analysis of sensitive biopharmaceutical analytes which exhibit surface adsorption artifacts as a means to improve recovery, and assay reproducibility. In this work, a well-conditioned LC system dedicated to RPLC analysis of proteins and peptides was shown to effectively recover a phosphorylated peptide only after surface passivation with an acid treatment. Waters ACQUITY Premier with MaxPeak HPS technology offers an off the shelf solution for enhanced chromatographic performance through improved analyte recovery and repeatability compared to more conventional LC technology.

References

1. D. T. Gjerde, C. P. Hanna, D. Hornby, Appendix 2: System Cleaning and Passivation Treatment, DNA Chromatography, Wiley-VCH Verlag GmbH & Co., 2002.
2. R. Day, “Passivating Stainless Steel in HPLC Systems,” Waters Lab Highlights, LAH 0376.

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- ACQUITY UPLC System <https://www.waters.com/514207>

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