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Application Note

Increasing Recovery of Phosphorylated Peptides Using ACQUITY Premier Technology Featuring MaxPeak High Performance Surfaces

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

Abstract

Phosphorylated groups in peptides can adsorb to metal surfaces of a liquid chromatography (LC) system and column hardware, resulting in peak tailing, reduced recovery, and poor reproducibility. These issues are exacerbated in analyses conducted at trace-levels due to diminished instrument response and/or significant surface adsorption of sensitive samples. Waters' ACQUITY Premier Solution featuring MaxPeak High Performance Surfaces (HPS) was evaluated in its ability to mitigate metal-induced surface adsorption of a phosphorylated peptide at trace levels. Using an RPLC-MS based technique, up to a 10-fold increase in MS detector response was observed for the phosphorylated peptide when using the ACQUITY Premier System with MaxPeak HPS technology in comparison to conventional hardware. The observed performance gain is partly attributed to the MaxPeak HPS technology's ability to significantly reduce tailing of metal sensitive analytes as well as increase recovery, evidence of which was observed in the area response of the phosphorylated peptide

(which increased proportionally with the MaxPeak HPS technology surface area). This study demonstrates how ACQUITY Premier Solution featuring MaxPeak High Performance Surfaces can improve data quality and productivity in the lab by improving recovery, peak shape, and reproducibility of metal-sensitive analytes.

Benefits

Waters' ACQUITY Premier Solution featuring MaxPeak High Performance Surfaces significantly improves recovery, peak shape, and reproducibility of phosphorylated peptides compared to conventional LC hardware and columns.

Introduction

Adsorption phenomena and strategies to reduce their impact on chromatographic performance continues to be an area of on-going interest. Adsorption artifacts, such as peak tailing, can increase assay variability and reduce the accuracy of quantitative analyses. These challenges are further exacerbated when working at trace-levels where adsorption artifacts can lead to diminished sample recovery and poor detector response of analytes. In certain instances, these adsorption artifacts can be attributed to interaction of analytes with metal surfaces. The underlying mechanism being that analytes bearing electron-rich moieties act as a Lewis base and adsorb in a non-covalent manner to electron deficient active sites on the metal surface of the LC and column hardware. Metal-induced adsorption artifacts such as this are of particular concern in the pharmaceutical industry where metal-sensitive moieties such as carboxylic acid and/or phosphate groups are commonly encountered in the composition of drug products. Efforts to reduce risk and untimely delays associated with method development or routine investigations require deployable solutions that can deliver reproducible results that accurately reflect drug composition.

Results and Discussion

The ACQUITY Premier System featuring MaxPeak High Performance Surfaces (HPS) technology is Waters' answer to challenges associated with adsorptive losses due to analyte/surface interaction. Based on experience

and established knowledge, Waters ACQUITY Premier product line featuring MaxPeak HPS technology is purposely engineered with a barrier layer to reduce non-specific adsorption of analytes for increased recovery, improved peak shape, and reproducibility of sensitive analytes. The objective of this study is to demonstrate the benefits of the ACQUITY Premier System in the analysis of phosphorylated peptides when working at trace levels using a reversed-phase liquid chromatography technique.

A comparison of three system configurations was made to examine how the ACQUITY Premier Solution featuring MaxPeak HPS technology improves sample recovery and peak shape compared to systems configured with conventional metal surfaces. These configurations include: a conventional LC system with a stainless-steel column, the same conventional LC system configured with an ACQUITY Premier Column, and the ACQUITY Premier Solution which is comprised of an ACQUITY Premier System configured with an ACQUITY Premier Column featuring MaxPeak HPS technology. In this study, a singly phosphorylated peptide (sequence VNQIGpTLSESIK, monoisotopic mass 1368.6776 Da) from the Waters MassPREP Phosphopeptide Standard Enolase (Waters P/N: 186003285 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186003285-massprep-phosphopeptide-standard-enolase.html>) was used to evaluate the performance of ACQUITY Premier with MaxPeak HPS technology. Phosphates ability to complex with metal-ions due to its electron-rich structure makes it an ideal probe to evaluate MaxPeak High Performance Surfaces technology and by extension the potential impact of ACQUITY Premier technology in the analysis of phosphate-containing analytes.

Briefly, a 2.0 µL injection of sample (3 pmol/µL) was separated under reversed phase LC conditions using a 1.85% B/min gradient (MP A: H_2O, 0.1% v/v FA, MP B: MeCN, 0.1% v/v FA). An ACQUITY QDa single quadrupole mass detector was used for data acquisition using selected ion recording (SIR) mode for maximum sensitivity (see figure 1 for instrument settings). Empower 3.0 (FR 4) was used for peak integration and data processing. An ACQUITY UPLC H-Class PLUS Bio System configured with an ACQUITY UPLC CSH C_{18} Column (P/N: 186005297 <https://www.waters.com/nextgen/us/en/shop/columns/186005297-acquity-uplc-csh-c18-column-130a-17--m-21-mm-x-100-mm-1-pk.html>) was used to represent a conventional LC system for comparison to the ACQUITY Premier Solution which was comprised of a ACQUITY Premier System configured with an ACQUITY Premier CSH Column (P/N: 186009488 < https://www.waters.com/nextgen/us/en/shop/columns/186009488-acquity-premier-peptide-csh-c18-column-130a-17--m-21-x-100-mm-1-.html>).

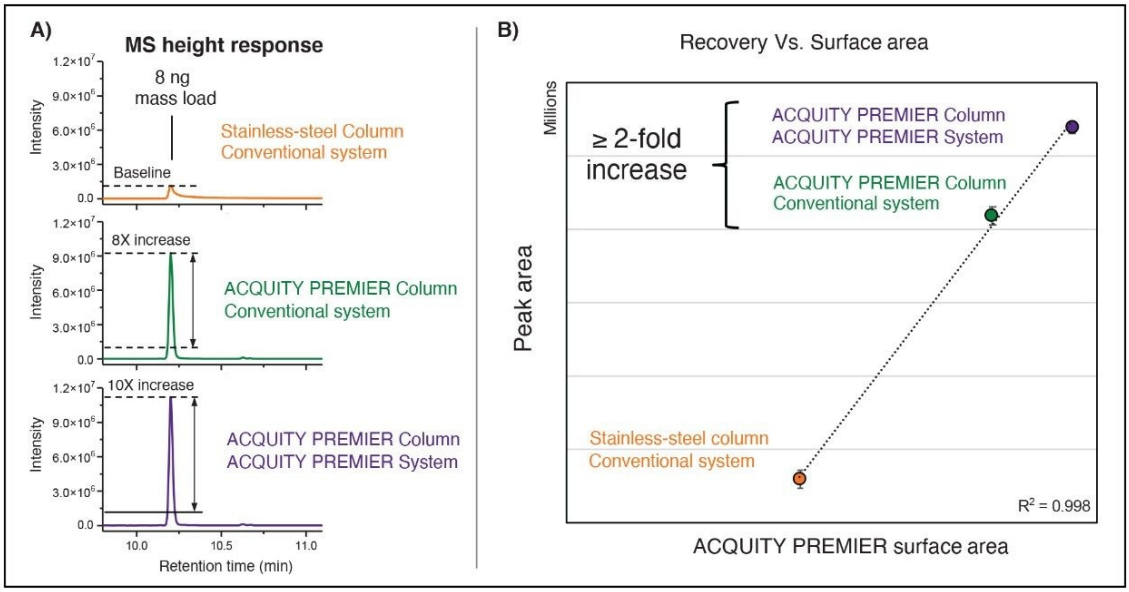

Figure 1. ACQUITY Premier with HPS technology. A) A selected ion recording (SIR) chromatogram for the $[M+2H]^{+2}$ charge state (m/z = 685.0) of a singly phosphorylated peptide (sequence: VNQIGpTLSESIK, average mass = 1369.4 Da) was used to determine MS-response for a conventional LC system and column (top panel), a conventional LC system configured with an ACQUITY Premier Column (middle panel), and an ACQUITY Premier System configured with an ACQUITY Premier Column (bottom panel). B) Mean peak area and standard deviation of corresponding SIRs for the phosphorylated peptide peak were calculated using a set of 3 replicate injections. ACQUITY QDa Mass Detector settings: Probe temperature = 600 °C, Capillary voltage = 1.5 kV, Cone voltage = 10 V, SIR = 685.0 m/z.

As shown in Figure 1A, at an on-column mass load of 8 ng, the phosphorylated peptide exhibited significant adsorption characteristics when the separation was performed on the conventional LC configured with a stainless-steel column (orange trace) resulting in peak tailing and severe broadening of the peak with a maximum signal observed at 1.1×10^6 ion counts. In contrast, tailing and peak shape were significantly improved when performing the same separation on the Waters ACQUITY Premier Column featuring MaxPeak HPS technology resulting in a 8-fold increase in signal intensity with a maximum signal response of 9.3×10^6 ion counts (green trace). Furthermore, a 10-fold increase in MS-response (ion counts = 1.1×10^7) was observed when the ACQUITY Premier Solution (ACQUITY Premier System configured with an ACQUITY Premier Column) was

used for the separation when compared to the conventional system using a stainless-steel column as shown in the violet trace of Figure 1A. The improved performance observed when using ACQUITY Premier Solution with MaxPeak High Performance Surfaces is attributed to its ability to not only reduce metal-induced adsorption artifacts such as tailing for sensitive analytes, but also improve sample recovery when working at trace levels. This is demonstrated in the peak area response as a function of surface area interaction as shown in Figure 1B. In this instance, the ACQUITY Premier Column with MaxPeak HPS technology exhibited the largest increase in sample recovery with a 2-fold increase in peak area when compared to the conventional LC system. This is attributed to the column hardware (e.g. frits and housing) having a significantly higher surface area contribution relative to the LC system surface area. However, maximum recovery of the phosphorylated analyte could only be achieved when using the ACQUITY Premier Solution (ACQUITY Premier System configured with an ACQUITY Premier Column) which recovered an additional 25% of the phosphorylated peptide in comparison to when using the ACQUITY Premier Column by itself. These results demonstrate how the ACQUITY Premier Solution with MaxPeak HPS technology, when deployed as an integrated solution for optimal chromatographic performance, enables efficient method development and accurate monitoring of sensitive analytes for improved productivity in the lab.

Conclusion

Non-specific adsorption of analytes when using LC systems configured with conventional metal hardware can lead to reduced sample recovery and poor peak shape. Waters' ACQUITY Premier Solution featuring MaxPeak High Performance Surfaces addresses the challenges associated with non-specific adsorption resulting in improved recovery, peak shape, and reproducibility of sensitive analytes. The performance gains realized by the ACQUITY Premier System with MaxPeak HPS technology enable scientists to increase productivity in the lab and mitigate risk through increased reproducibility, recovery, and robustness of assays performed in the development and manufacturing of biopharmaceutical drug products.

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ACQUITY Premier Solution <https://www.waters.com/waters/nav.htm?cid=135071970>

ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

Empower Chromatography Data System <https://www.waters.com/10190669>

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