

## Enhancing Phosphopeptide Quantitation Using ACQUITY Premier Peptide CSH C<sub>18</sub> Columns

---

Jennifer M. Nguyen, Susan C. Rzewuski, Matthew A. Lauber

Waters Corporation

---

### Abstract

During LC separations, many analytes, especially those bearing a negative charge, can adsorb to electron-deficient metal oxide surfaces and thereby be challenging to robustly analyze. In the case of peptides, these compounds may intrinsically contain acidic, negatively charged residues like aspartic acid and glutamic acid. Further still, post-translational modifications such as phosphorylation can add acidic character to peptides and exacerbate adsorptive losses.<sup>1</sup>

Some common methods to circumvent this challenge are the use of mobile phases additives or chelators. Strong acids have been observed to improve the recovery of phosphorylated compounds, yet this method can be detrimental to those analyses requiring MS detection.<sup>2,3</sup> Use of chelating agents, which can also help prevent metal-analyte adsorption,<sup>4</sup> has drawbacks in the form of reduced MS intensity or solubility issues.<sup>5</sup>

Recent studies have shown that ACQUITY Premier Columns and their MaxPeak High Performance Surfaces (HPS) can be used to enhance the recovery and reproducibility of analytes such as peptides.<sup>6</sup> This technology has been demonstrated to mitigate metal-analyte interactions that could aggravate sample loss and has been proven to be a more effective hardware technology for this purpose when compared to titanium.<sup>7</sup> In this

---

application note, we further demonstrate the potential of ACQUITY Premier Columns for use in the analysis of phosphorylated peptides, which might be of interest for proteomics analyses or studies involving kinase inhibitors.<sup>8</sup>

## Benefits

- Improved recovery versus conventional column technologies
- Minimal to no column conditioning required
- Better reproducibility and method robustness
- Higher quality MS spectra

---

## Introduction

In chromatography, the adsorption of analytes to metal surfaces can be very problematic and it can contribute to peak tailing, low analyte recovery, and poor reproducibility. Loss of analyte as a result of these metal ion mediated interactions can greatly affect results. Lower mass loads have been shown to be difficult to recover due to these adsorptive effects, which can be detrimental for assays that require low limits of detection and quantification.<sup>9</sup>

Quantitative bioanalysis of phosphopeptides is one such application where high recovery is necessary. Because phosphorylation on proteins is an essential post-translational modification (PTM) that modulates the cell signaling in many biological pathways, it is extremely important to characterize, detect, and quantify these PTMs.<sup>10</sup> Often times, in order to detect phosphorylation, the protein is digested to its phosphopeptides and monitored for phosphorylation on serine, threonine, and tyrosine residues.<sup>11</sup> However, phosphopeptides are often not as abundant as non-phosphorylated peptides. Enrichment is thus required to make analysis possible.<sup>10</sup> In addition, phosphopeptides are known to adsorb to metals, making them even more difficult to detect and quantify.<sup>1</sup>

In this application note, we investigate the recovery and quantitative performance of phosphorylated and non-phosphorylated peptide analysis using ACQUITY Premier Peptide CSH C<sub>18</sub> columns, which feature MaxPeak High Performance Surfaces (HPS) Technology. This hybrid organic/inorganic surface technology minimizes adsorptive losses by blocking interactions to metal surfaces.<sup>9,12</sup> Here, we compare the initial performance of

this MaxPeak Premier Technology versus conventional columns, where the MaxPeak Premier Columns demonstrate improved recovery and reproducibility, even after both columns were conditioned. In addition, we performed a quantitative comparison based on plate reader quantitation of collected fractions, in which the MaxPeak Premier Technology displayed nearly full recovery of a doubly phosphorylated peptide upon even its first injection. A detailed study of these column effects was made possible through the use of an ACQUITY Premier System, which eliminated metal interactions in the LC system.

---

## Experimental

### Initial Column Performance

#### Sample Preparation

A lyophilized pellet of Angiotensin I (sequence of DRVYIHPFHL) was reconstituted in 0.1% TFA in water with 10% DMSO to give a concentration of 771 pmol/ $\mu$ L and further diluted to 60 pmol/ $\mu$ L to make the three peptide mixture.

A lyophilized pellet of Enolase T37 (sequence of YPIVSIEDPFAEDDWEAWSHFFK) was reconstituted in 0.1% TFA in water with 10% DMSO to yield a final concentration of 353 pmol/ $\mu$ L and further diluted to 60 pmol/ $\mu$ L to make the three peptide mixture.

A lyophilized pellet of doubly phosphorylated insulin receptor (sequence of TRDlpYETDpYYRK) and a molecular weight of 1782.6 Da was reconstituted in 0.1% formic acid in water to yield a 500 pmol/ $\mu$ L concentration sample for use with conditioning steps and further diluted to 60 pmol/ $\mu$ L to make the three peptide mixture.

Equal volumes of 60 pmol/ $\mu$ L Angiotensin I, Enolase T37, and doubly phosphorylated insulin receptor peptide were used to prepare a 1:1:1 equimolar mixture having a final concentration for each peptide that was approximately 20 pmol/ $\mu$ L.

#### Data Treatment and Analysis

Three ACQUITY UPLC Peptide CSH Columns were compared to three ACQUITY Premier Peptide CSH C<sub>18</sub> Columns. The initial column performance (peak areas, gradient peak capacities, and retention times) from the first four injections was evaluated and the averages taken before conditioning the column with a high mass load

---

of peptide and running a fifth injection. The relative amount of recovered peptide (%) was estimated from the observed peak areas from the UV chromatogram.

## Spectrofluorometric Quantitation of 5-FAM Labeled Peptide

### Sample Preparation and Calibration Curve Generation

For use as a standard in spectrofluorometry, a peptide labelled with 5-carboxyfluorescein (5-FAM), a dye used to impart fluorescence properties to biomolecules, with a sequence of 5-Fam-AKRRRLpSpSLRA and molecular weight of 1831.9 Da was serially diluted to a final concentration of 1 pmol/ $\mu$ L and 0.1 pmol/ $\mu$ L using a 79 to 21 percent solution of mobile phase A to mobile phase B. Calibration curves with a linear dynamic range of 0.02 pmol to 0.80 pmol mass loads were generated using a Gemini XPS spectrofluorometer from Molecular Devices (San Jose, CA). Analyses were performed with excitation and emission wavelengths of 443 nm and 516 nm using SoftMax Pro 6.5.1 for data acquisition and analysis.

### Data Treatment and Analysis

Duplicate sets of ACQUITY UPLC Peptide CSH Columns were compared against ACQUITY Premier Peptide CSH C<sub>18</sub> Columns. Upon initial use of each set, four separations of 5-FAM labelled peptide were performed. Fractions containing 5-FAM labelled peptide were collected during the fourth injection of each set, and the resultant peptide recovery was determined through spectrofluorometric quantitation. All previous runs of 5-FAM labelled peptide were assigned a percent recovery value based on ratios of UV peak area and the concentration determined for the fraction collected from the fourth injection/separation.

## LC Conditions

LC system:	ACQUITY Premier (QSM)
Detection:	UV at 214 nm
Vials:	QuanRecovery with MaxPeak HPS Vials (p/n: 186009186)
Column(s):	ACQUITY UPLC Peptide CSH C <sub>18</sub> Column, 130Å, 1.7 $\mu$ m, 2.1 mm x 50 mm (p/n:

---

186006936)

ACQUITY Premier Peptide CSH C<sub>18</sub> Column,  
130Å, 1.7 µm, 2.1 x 50 mm (p/n: 186009487)

Column temp.: 60 °C

Sample temp.: 4 °C

Injection volume: 1.0 µL (sample), 10.0 µL (conditioning)

Flow rate: 0.2 mL/min

Mobile phase A: 0.1% (v/v) Formic acid in water

Mobile phase B: 0.09% (v/v) Formic acid in acetonitrile

## Gradient Table

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.20	99.5	0.5	6
12.00	0.20	60.0	40.0	6
14.00	0.20	20.0	80.0	6
14.01	0.20	99.5	0.5	6
17.00	0.20	99.5	0.5	6

## MS Conditions

MS system: Xevo G2-XS QTof

Ionization mode: ESI positive, resolution

Acquisition range:	100–2000 <i>m/z</i>
Capillary voltage:	2.5 kV
Sampling cone:	80 V
Source offset:	80 V
Source temp.:	120 °C
Desolvation temp.:	500 °C
Desolvation gas:	800 L/h

## Data Management

Chromatography and MS software:	Empower 3 FR4 or MassLynx 4.1 for data acquisition; UNIFI 1.8 for data analysis
---------------------------------	--

---

## Results and Discussion

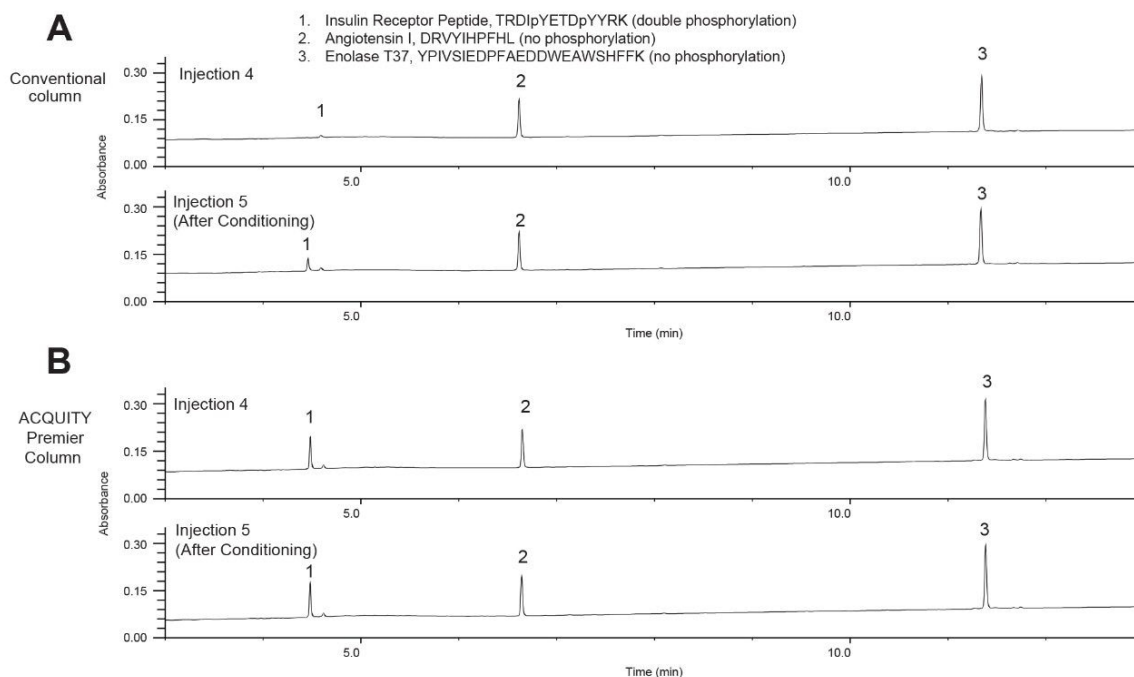
### Initial Column Performance

In this investigation, we sought to evaluate the separation and recovery of phosphopeptides using the MaxPeak Premier Technology. Phosphopeptides are known to adsorb to metal surfaces such as stainless steel or titanium. Thus, we analyzed the LC-UV-MS separations of an equimolar mixture of peptides consisting of a doubly phosphorylated insulin receptor peptide, angiotensin I, and enolase T37 using either conventional ACQUITY UPLC Peptide CSH C<sub>18</sub> Columns or ACQUITY Premier Peptide CSH C<sub>18</sub> Columns packed with the same lot of

stationary phase.

Typically, reversed-phase separations of peptides are performed with acidic mobile phase modifiers such as formic acid (FA), difluoroacetic acid (DFA), or trifluoroacetic acid (TFA). Since formic acid is a weaker ion pairing reagent, it is preferred for MS analyses.<sup>13</sup> For peptide separations, formic acid separations might also show a higher degree of susceptibility to allowing metal binding interactions. For this reason, mobile phases of 0.1% (v/v) modified water and 0.09% (v/v) modified acetonitrile mobile phases containing formic acid (FA) were employed. Initial column performance (peak areas, gradient peak capacities, and retention times) from the first four injections was evaluated before conditioning the column with a high mass load of peptide and running a fifth injection. Injecting a high load of sample is often used as a way to condition the column or LC flow path, whereby the large quantity of sample will saturate binding sites within the column or LC. Tests were performed at a column temperature of 60 °C and a flow rate of 0.2 mL/min on an ACQUITY Premier System configured with MaxPeak High Performance Surfaces. This made it possible to make more direct observations on the effects of column hardware. Representative UV chromatograms resulting from the fourth and fifth injections immediately after high mass load column conditioning are shown in Figure 1. Gradient peak capacities and peak areas for angiotensin I and enolase T37 were essentially equivalent across the first four injections for all columns. However, with regards to the doubly phosphorylated insulin receptor peptide, there was practically no recovery of this peptide upon the initial use of conventional columns (Figure 1A).

In contrast, ACQUITY Premier Columns demonstrated reproducible performance over all injections regardless of column conditioning (Figure 1B). Post-conditioning changes in average peak areas, gradient peak capacities, and retention times were less than 3%. After conditioning the conventional columns, an improvement in the recovery of the doubly phosphorylated peptide could be achieved, yet this peak area was still only 39% of that of the ACQUITY Premier Columns. This suggests that further conditioning is required or that full recovery may not be possible using conventional column technology.



*Figure 1. UV chromatograms of the fourth injection (before column conditioning) and fifth injection (after column conditioning) of an equimolar mixture of angiotensin I, enolase T37, and doubly phosphorylated insulin receptor obtained using (A) an ACQUITY UPLC CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column or (B) an ACQUITY Premier CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column. Separations were performed with an ACQUITY Premier System using a flow rate of 0.2 mL/min, column temperature of 60 °C, FA-modified mobile phases, and 20 pmol mass loads.*

Due to its two phosphorylation sites, the recovery of the insulin receptor peptide may have suffered due to the increased negative charge of these groups and a higher degree of adsorption to metal hardware surfaces. It might seem by these results that angiotensin I and enolase T37 were unaffected by choice in column hardware. Looking at mass spectra collected during the separations showed something different. Indeed, mass spectrometric analysis was also assessed with the three-peptide mixture as separated on the conditioned columns. Analyses were performed with electrospray ionization and a Xevo G2-XS QToF. While the data from the TICs correlated with the UV data in terms of peptide peak area, we found contrast in the peptide mass spectra, where higher quality MS data were readily obtained with the ACQUITY Premier Column. Separations using conventional columns yielded a high abundance of iron adducts for all charge states of each peptide. As exemplified in the mass spectra of enolase T37 (Figure 2A), these separations were populated with iron adducted



ions that are presumably leached from the metal hardware surfaces. In the case of the 4+ charge state of enolase T37, the iron adducted peak is the most abundant ion signal (Figure 2A). The level of iron adducts in the 3+ charge state was 5.9%, at the 2+ charge state was 9.5%, and at the 4+ charge state, was over two-fold higher than that of the main peak. In comparison, separations performed with ACQUITY Premier Peptide CSH C<sub>18</sub> Columns reduced iron adducts by 80–90% (Figure 2B).

In addition, we observed that the ACQUITY Premier Columns yielded lower charge state ions. Presumably, it could be that the presence of iron ions can affect the desolvation gas efficiency and ionization of peptides, thus favoring higher charge states. The simple addition of one iron atom versus one proton would also skew charge state distributions wherever iron ions are present during the electrospray process. Our results from the conventional columns gave a relative abundance of 19.5% for the 2+ charge state of enolase T37, while for the ACQUITY Premier Columns, a 29% abundance was observed. When comparing the 2+ charge state relative to the 3+ charge state, the ACQUITY Premier Columns were 50% higher in abundance at the lower charge state versus the conventional columns. While low-level metal contaminants might not be seen here to affect peptide separations, it is clear that there can be an influence on the quality of peptide mass spectra.

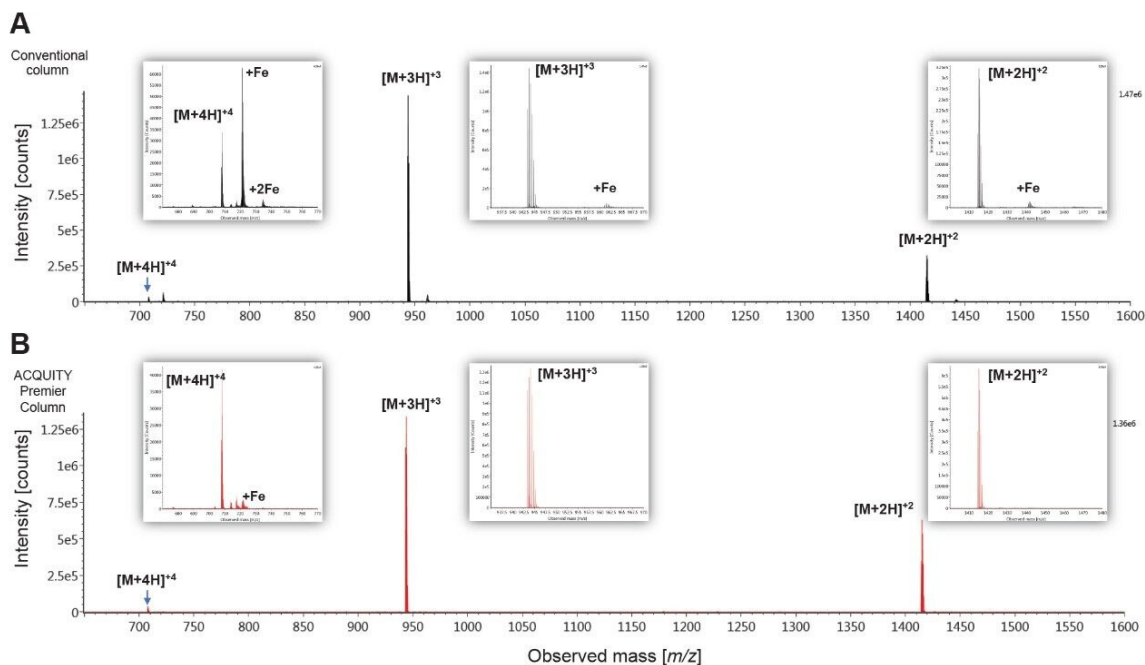


Figure 2. Mass spectra of enolase T37 from a separation of an equimolar mixture of angiotensin I, enolase T37, and doubly phosphorylated insulin receptor peptide obtained using a previously conditioned (A) ACQUITY UPLC CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column or (B) ACQUITY Premier CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column. Separations were performed with an ACQUITY Premier System using a flow rate of 0.2 mL/min, column temperature of 60 °C, FA-modified mobile phases, and 20 pmol mass loads.

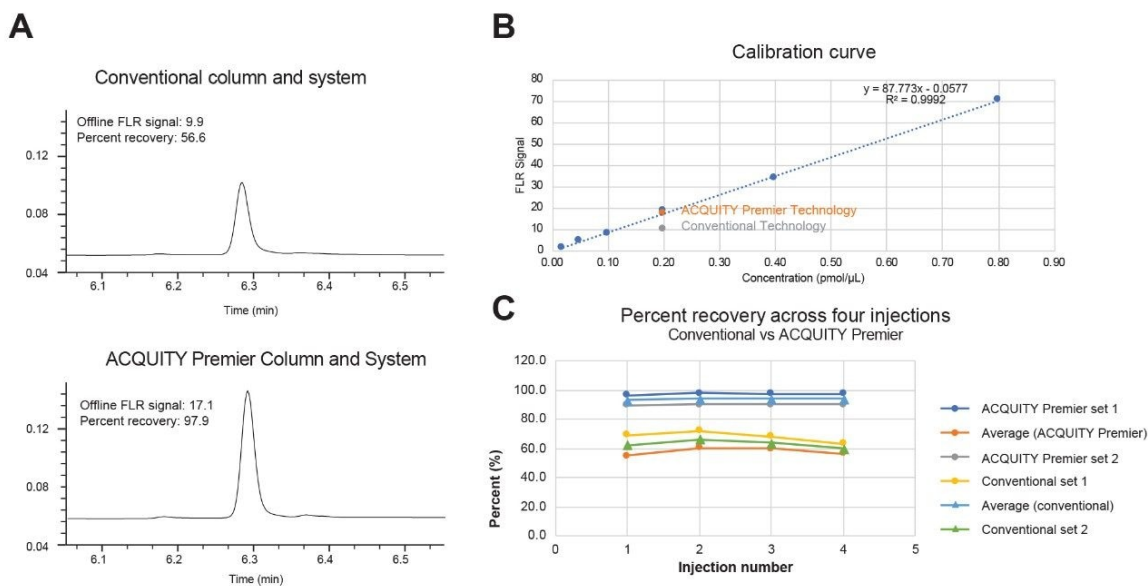
## Spectrofluorometric Quantitation of 5-FAM Labeled Peptide

Quantification of peptides via LC-MS is an established technique for the discovery and development of biotherapeutics as well as the discovery of small molecule drugs that can alter phosphorylation cascades. Herein, we have seen that peptides with modifications granting additional charge have proven difficult to recover. As seen in the case of the doubly phosphorylated insulin receptor peptide, modifications such as a negatively charged phosphate group can cause a peptide to be extremely difficult to recover due to its affinity to metals. For this part of the study, we sought to quantify these benefits of the MaxPeak Premier Technology.

To accurately measure and compare the amount of peptide recovered, we employed spectrofluorometric detection to generate standard curves for quantification through the use of an offline plate reader assay.

Originally, we planned to continue with the doubly phosphorylated insulin receptor for quantitative analysis of recovery, but this peptide did not contain a strong fluorophore and was unstable and insoluble in solution when fluorescently tagged. Therefore, a more stable doubly phosphorylated peptide labeled with 5-FAM was used. Four injections of 5-FAM labeled peptide were first performed on a conventional LC system and conventional column. Fractions containing 5-FAM labeled peptide were collected during the fourth injection for two different sets of unique components. This approach was repeated with ACQUITY Premier System components paired with ACQUITY Premier Columns.

Peptide recovery as seen in the analysis of the phosphorylated insulin receptor peptide translated to the 5-FAM labeled phosphorylated peptide. Better recovery was detected using the MaxPeak Premier Technology. Interestingly, the extent of recovery differences was not as pronounced for this phosphopeptide versus the one used in the previous experiment and with the same chromatographic conditions (Figure 3A). A possible explanation for this result could be due to the phosphorylation occurring on a different amino acid, serine, in the 5-FAM labeled peptide instead of tyrosine, where the negatively charged phosphate group would be more sterically hindered. It could also be that the doubly phosphorylated insulin receptor peptide possesses a higher negative surface potential than the 5-FAM labeled peptide that facilitates stronger metal affinity and chromatographic adsorption.<sup>14</sup> Moreover, there could be differences in conformational effects that might allow one peptide to engage in surface adsorption more readily than the other.



*Figure 3. (A) UV chromatograms of the fourth injection of 5-FAM labeled peptide as obtained using a conventional LC system and an ACQUITY UPLC CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column or an ACQUITY Premier System and ACQUITY Premier CSH C<sub>18</sub>, 1.7  $\mu$ m, 2.1 x 50 mm Column. (B) Calibration curve and quantitative measurement of the fourth 5-FAM labeled peptide fraction as generated through offline spectrofluorometric analyses across six different concentrations (0.02, 0.05, 0.10, 0.20, 0.40, and 0.80 pmol/ $\mu$ L). (C) Percent recoveries for individual and average sets across the four injections of 5-FAM labeled peptide as calculated based on ratios of UV peak area and the concentration determined for the fraction collected from the fourth injection. Separations were performed using a flow rate of 0.2 mL/min, column temperature of 60 °C, FA-modified mobile phases, and 20 pmol mass loads.*

Calibration curves as seen in Figure 3B were used to calculate the percent recovery of the 5-FAM labeled peptide. For the data shown in Figure 3A, the first set of the ACQUITY Premier Column and ACQUITY Premier LC components gave almost full recovery of the phosphorylated peptide; a recovery measurement of 97.9% was observed. This was 73% more than that of the conventional columns used with a conventional LC system. Furthermore, we determined that there was little-to no-change in percent recoveries across four injections for either replicate 1 or 2 of analyzing ACQUITY Premier Columns on an ACQUITY Premier System. Average recoveries ranged from 93.5% to 94.2% with this new LC setup (Figure 3C). In contrast, the conventional columns paired with a conventional LC system demonstrated lower and more variable peptide recoveries that

ranged from 60.1% to 66.4%. That very high recovery of the phosphorylated peptide was obtained with MaxPeak Premier Technology on even the first injection demonstrates the utility of more inert chromatographic surfaces for the analysis of phosphorylated peptides.

---

## Conclusion

Here, we demonstrate that an ACQUITY Premier Peptide CSH C<sub>18</sub> Column with MaxPeak High Performance Surfaces (HPS) Technology provides substantial improvements in recovery and reproducibility for peptides with acidic modifications, such as phosphorylation. This data reconfirms prior observations that the surface barrier provided by MaxPeak HPS mitigates metal ion mediated adsorption. Unlike the conventional columns, the ACQUITY Premier Columns gave consistent performance upon their first time use and very first injection.

Analogous to previous observations for acidic small molecule compounds, we also found that cleaner MS spectra could be achieved for not only the doubly phosphorylated peptide, but for all peptides in our study.<sup>12</sup> While the mass spectra from conventional columns gave highly contaminated iron adducted ions, ACQUITY Premier Columns displayed little to no iron adducts.

In all, these results show that MaxPeak Premier Technology can be advantageously applied to enhance the chromatographic performance of peptide-based assays, most especially those aiming to identify and quantify phosphorylation. Undoubtedly, this technology could be used to improve the robustness and sensitivity of bioanalytical assays for phosphopeptides or other negatively charged molecules.

---

## References

1. Tuytten; R.; Lemièrè; F.; Witters; E.; Van Dongen, W.; Slegers, H.; Newton, R. P.; *et al.* Stainless-Steel Electropray Probe: A Dead End for Phosphorylated Organic Compounds. *J. Chromatogr., A*. 2006, 1104, 209-21. doi:10.1016/j.chroma.2005.12.004.
  2. Asara, J. M.; Allison, J. Enhanced Detection of Phosphopeptides in Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Using Ammonium Salts. *J. Am. Soc. Mass Spectrom.* 1999, 10, 35-
-

44. doi:10.1016/S1044-0305(98)00129-9.
3. Kjellstrom, S.; Jensen, O. N. Phosphoric Acid as a Matrix Additive for MALDI MS Analysis of Phosphopeptides and Phosphoproteins. *Anal. Chem.* 2004, 76, 5109–17. doi:10.1021/ac0400257.
4. Birdsall, R. E.; Kellett, J.; Yu, Y. Q.; Chen, W. Application of Mobile Phase Additives to Reduce Metal-Ion Mediated Adsorption of Non-Phosphorylated Peptides in RPLC/MS-based Assays. *J. Chromatogr., B: Anal. Technol. Biomed. Life Sci.* 2019, 1126–1127, 121773. doi:10.1016/j.jchromb.2019.121773.
5. Lough, J.; Mills, M. J.; Maltas, J. Analyte Adsorption in Liquid Chromatography Valve Injectors for Samples in Non-Eluting Solvents. *J. Chromatogr., A.* 1996, 726, 67–75.
6. Birdsall, R. E.; Kellett, J.; Ippoliti, S.; Ranbaduge, N.; Shion, H.; Yu, Y. Q. Increasing Chromatographic Performance of Acidic Peptides in RPLC-MS-based Assays with ACQUITY Premier Featuring MaxPeak HPS Technology. Waters Application Note, 720007003 <<https://www.waters.com/nextgen/us/en/library/application-notes/2020/increasing-chromatographic-performance-of-acidic-peptides-in-rplc-ms-based-assays-with-acquity-premier-featuring-maxpeak-hps-technology.html>> , 2020.
7. DeLoffi, M.; Nguyen, J. M.; Izzo, G. S.; Lauber, M.; Savaria, M. Improved Chromatographic Performance with an ACQUITY Premier Peptide C<sub>18</sub> Column Versus a Titanium-lined C<sub>18</sub> Column Technology. Waters Application Brief, 720007022 <<https://www.waters.com/nextgen/us/en/library/application-notes/2020/improved-chromatographic-performance-with-a-premier-peptide-c18-column-versus-a-titanium-lined-c18-column-technology.html>> , 2020.
8. Ji, L.; Wu, J. H.; Luo, Q.; Li, X.; Zheng, W.; Zhai, G.; *et al.* Quantitative Mass Spectrometry Combined with Separation and Enrichment of Phosphopeptides by Titania Coated Magnetic Mesoporous Silica Microspheres for Screening of Protein Kinase Inhibitors. *Anal. Chem.* 2012, 84, 2284–91. doi:10.1021/ac202897u.
9. Lauber, M.; Walter, T. H.; Gilar, M.; DeLano, M.; Boissel, C. A.; Smith, K.; *et al.* Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces. Waters White Paper, 720006930EN <<https://www.waters.com/waters/library.htm?cid=511436&lid=135074404&lcid=135074403>> , 2020.
10. Cheng, L. C.; Li, Z.; Graeber, T. G.; Graham, N. A.; Drake, J. M. Phosphopeptide Enrichment Coupled with Label-Free Quantitative Mass Spectrometry to Investigate the Phosphoproteome in Prostate Cancer. *J. Vis. Exp.* 2018. doi:10.3791/57996.

11. McLachlin, D. T.; Chait, B. T. Analysis of Phosphorylated Proteins and Peptides by Mass Spectrometry. *Current Opinion in Chemical Biology*. 2001, 5, 591–602. doi:10.1016/s1367-5931(00)00250-7.
  12. Smith, K. M.; Wilson, I. D.; Rainville, P. D. Sensitive and Reproducible Mass Spectrometry-Compatible RP-UHPLC Analysis of Tricarboxylic Acid Cycle and Related Metabolites in Biological Fluids: Application to Human Urine. *Anal. Chem.* 2020. doi:10.1021/acs.analchem.0c03863.
  13. Lauber, M. A.; Koza, S. M.; McCall, S. A.; Alden, B. A.; Iraneta, P. C.; Fountain, K. J. High-Resolution Peptide Mapping Separations with MS-friendly Mobile Phases and Charge-Surface-Modified C<sub>18</sub>. *Anal. Chem.* 2013, 85, 6936–44. doi:10.1021/ac401481z.
  14. Vallee, A.; Humblot, V.; Pradier, C. M. Peptide Interactions with Metal and Oxide Surfaces. *Acc. Chem. Res.* 2010, 43, 1297–306. doi:10.1021/ar100017n.
- 

## Featured Products

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

[Xevo G2-XS QToF Quadrupole Time-of-Flight Mass Spectrometry <https://www.waters.com/134798222>](https://www.waters.com/134798222)

[MassLynx MS Software <https://www.waters.com/513662>](https://www.waters.com/513662)

[Empower Chromatography Data System <https://www.waters.com/10190669>](https://www.waters.com/10190669)

[UNIFI Scientific Information System <https://www.waters.com/134801648>](https://www.waters.com/134801648)

[MaxPeak Premier Columns <https://www.waters.com/waters/nav.htm?&cid=135074078>](https://www.waters.com/waters/nav.htm?&cid=135074078)

720007211, March 2021

© 2022 Waters Corporation. All Rights Reserved.

[Terms of Use](#)

[Privacy](#)

[Trademarks](#)

[Sitemap](#)

[Careers](#)

[Cookies](#)

[Cookie Preferences](#)