# Efficient Recovery and Analysis of Phosphopeptides using Improved Chromatographic Surface Chemistry and a New Multi-Reflecting-TOF Mass Spectrometer

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## Introduction

- Phosphopeptide detection by LC-MS is challenging because phosphate groups strongly interact with the metal surfaces within the LC flow path, leading to analyte adsorption, reduced recovery, and peak tailing.

- Pre-passivation of the LC flow path with metal chelators (*e.g.*, ETDA[1], citric acid[2]) can reduce phosphate-metal interactions, but these steps increase workflow complexity, are time-consuming and often provide limited effectiveness.

- High-Performance Surfaces (HPS)[3] reduce analyte-metal interactions by incorporating a highly effective inorganic/organic surface barrier on the wetted hardware surfaces.

- This study compares HPS-modified 300 µm column hardware with standard stainless-steel hardware to assess improvements in phosphopeptide recovery, chromatographic peak shape, and LC-MS detection performance.
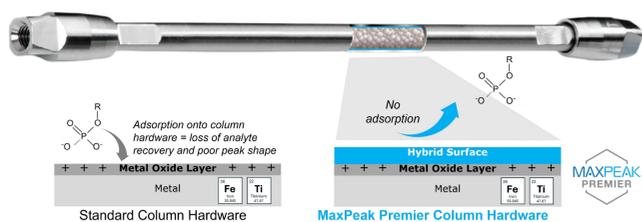
**Figure 1.** MaxPeak™ Premier Columns use a covalently bonded hybrid surface to minimize analyte–metal interactions, enhancing recovery and sensitivity.

## Methods
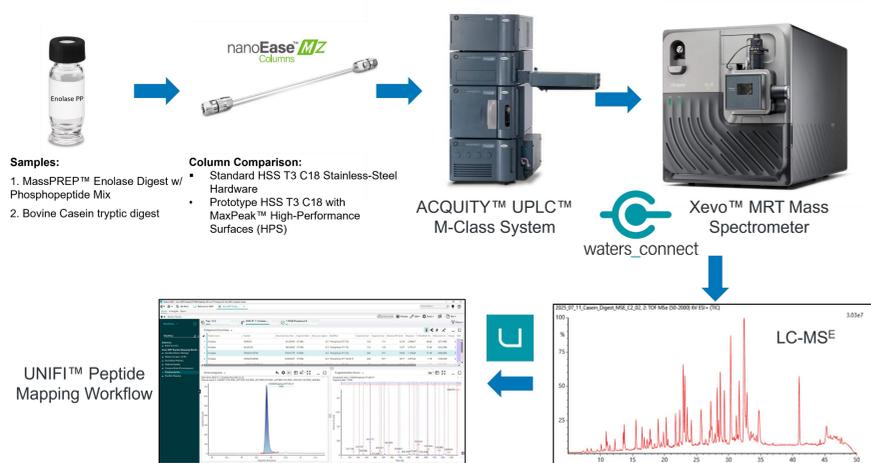
**Figure 2.** Experimental workflow comparing the phosphopeptide performance of the HPS-prototype 300 µm column with the standard stainless-steel column using an ACQUITY M-Class System equipped with a low-flow ESI probe interfaced to the Xevo MRT Mass Spectrometer. Mobile Phases: A: 0.1% Formic Acid in water, B: 0.1% Formic Acid in acetonitrile. Gradient: 1-40% mobile phase B over 30 min. Flow Rate: 8.0 µL/min

| Peptide ID | Peptide Sequence | Monoisotopic Mass [M+H]+ (Da) | m/z [M+2H]2+ |
|---|---|---|---|
| T18 (1P) | NVPL**pY**K | 813.3912 | 407.1995 |
| T19 (1P) | HLADL**pS**K | 863.4028 | 432.2053 |
| T43 (1P) | VNQIQ**pT**LESESIK | 1368.6776 | 684.8428 |
| T43 (2P) | VNQIQTL**pSE**p**S**IK | 1448.6439 | 724.8259 |

**Table 1**. Peptide sequences, monoisotopic masses, and doubly charged m/z values of yeast enolase–derived phosphopeptides (T18p, T19p, T43p, T43pp) in the MassPREP Enolase Digest Phosphopeptide Mix.

## Conclusions

- The HPS-prototype 300 µm columns enabled higher phosphopeptide recovery without requiring metal-chelating pre-passivation.

- The enolase and casein results confirm improved phosphopeptide recovery and superior chromatographic performance.

- The Xevo MRT Mass Spectrometer delivers ~100k resolving power and supports MS/MS acquisition rates up to 100 Hz with sub-ppm mass accuracy.

- The Xevo MRT Mass Spectrometer combined with the MaxPeak Premier microflow columns provides a high-sensitivity, high-recovery workflow ideal for phosphoproteomics.

## Results: enolase and casein tryptic digests
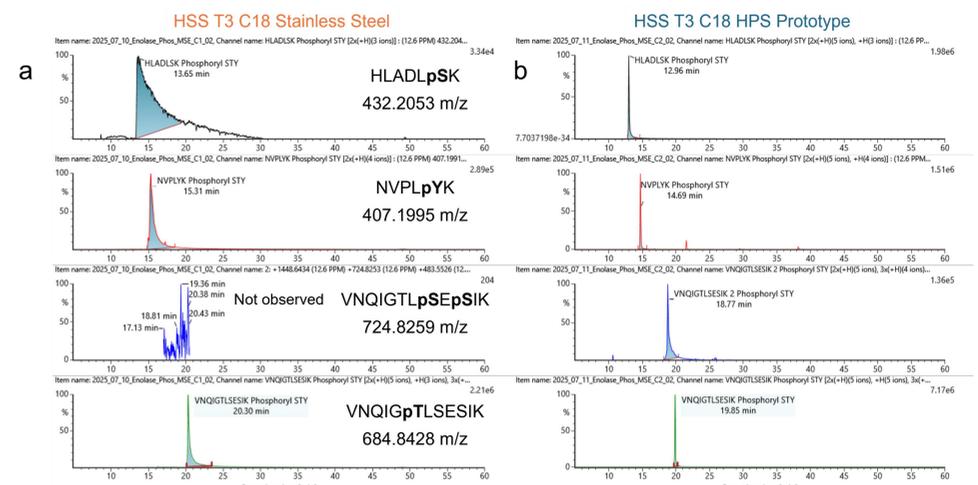
**Figure 3.** Extracted ion chromatograms of yeast enolase–derived phosphopeptides separated on (a) stainless-steel and (b) MaxPeak Premier (HPS) Prototype Hardware. Columns were pre-conditioned with three injections of 1.0 pmol MassPREP BSA digest prior to analysis of the 1.0 pmol phosphopeptide standard.
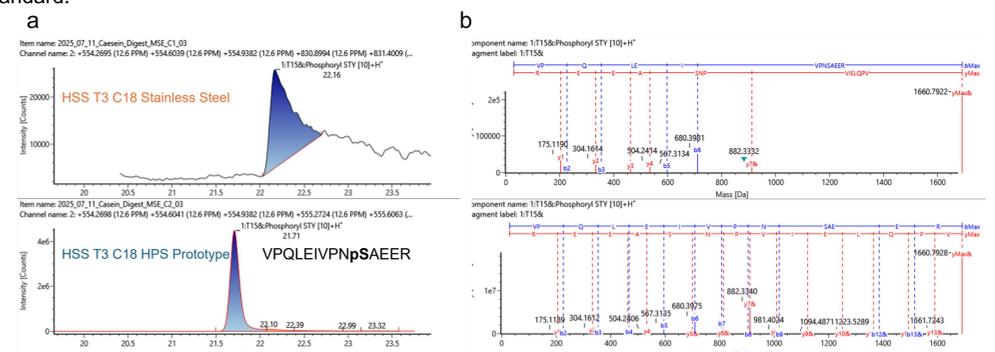
**Figure 4.** Enhanced phosphopeptide recovery improves chromatographic peak shape, as shown by (a) extracted ion chromatograms of the αS1-casein T15 singly phosphorylated peptide (VPQLEIVPN**pS**AEER), and yields (b) more sequence-informative fragment ions in elevated-energy mass spectra.
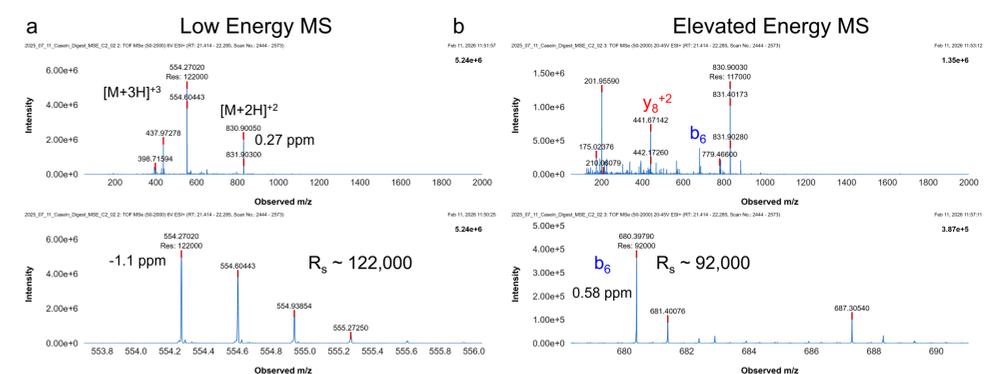
**Figure 5.** Corresponding (a) low-energy and (b) elevated-energy mass spectra of the αS1-casein T15 singly phosphorylated peptide (VPQLEIVPN**pS**AEER). Expanded views for each spectrum are provided below, including annotated mass spectral resolution values and ppm-level mass accuracy for key fragment ions.
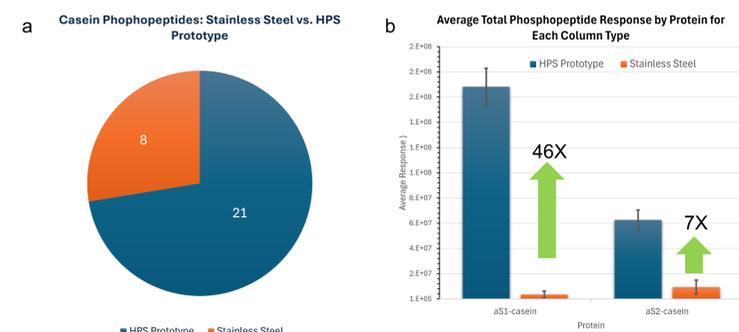
**Figure 6.** (a) Pie chart comparing the total number of unique phosphopeptides from αS1- and αS2-casein detected across triplicate analyses using stainless-steel and MaxPeak Premier (HPS) Prototype Hardware. (b) Bar chart showing the average total phosphopeptide response for each column type, highlighting the substantially improved phosphopeptide recovery achieved with MaxPeak Premier HPS hardware.

## References

1. Liu S, *et al.* Rapid Commun Mass Spectrom. 2005;19(19):2747-56
2. Birdsall, R.E., *et al.* Journal of Chromatography B 1126–1127 (2019) 121773
3. DeLano, M., *et al.* Anal. Chem. 2021, 93, 5773–5781

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