Analysis of phosphorylated peptides using a bioinert YMC-Accura Triart C18 column

This application note demonstrates the benefit of specifically designed column hardware for biomolecules, which contain phosphate group(s). This includes:

- **(Phospho-)peptides**
- **Nucleotides**
- **Oligonucleotides**

The YMC-Accura column hardware has been designed to eliminate any interaction between sample and stainless steel due to a strict coating of column body and frit. It allows sharp peaks, stable recoveries and eliminates consequential carry-over effects. This makes it a great choice for working at trace-levels.

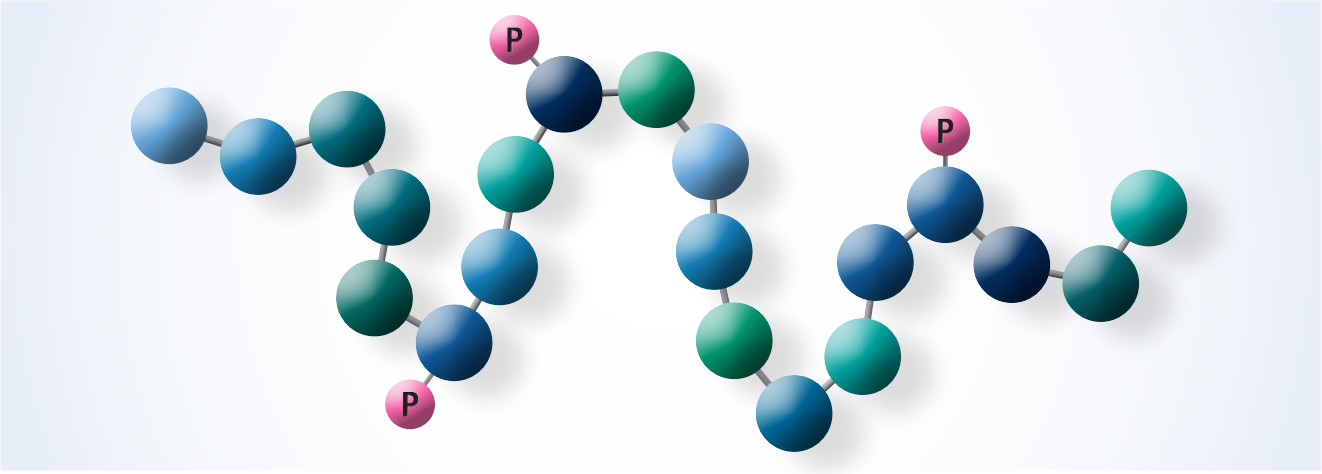

Figure 1: Phosphorylated peptide chain.

As an example, this application note shows results obtained with standard column hardware in comparison with the bioinert coated YMC-Accura Triart C18 column. To demonstrate the beneficial effect four phosphorylated peptides (figure 2) have been selected. To eliminate the influence of any potential interaction between these critical analytes and any metal parts in the LC system, all measurements were performed on the new and fully bioinert LC system Nexera XS inert from Shimadzu.

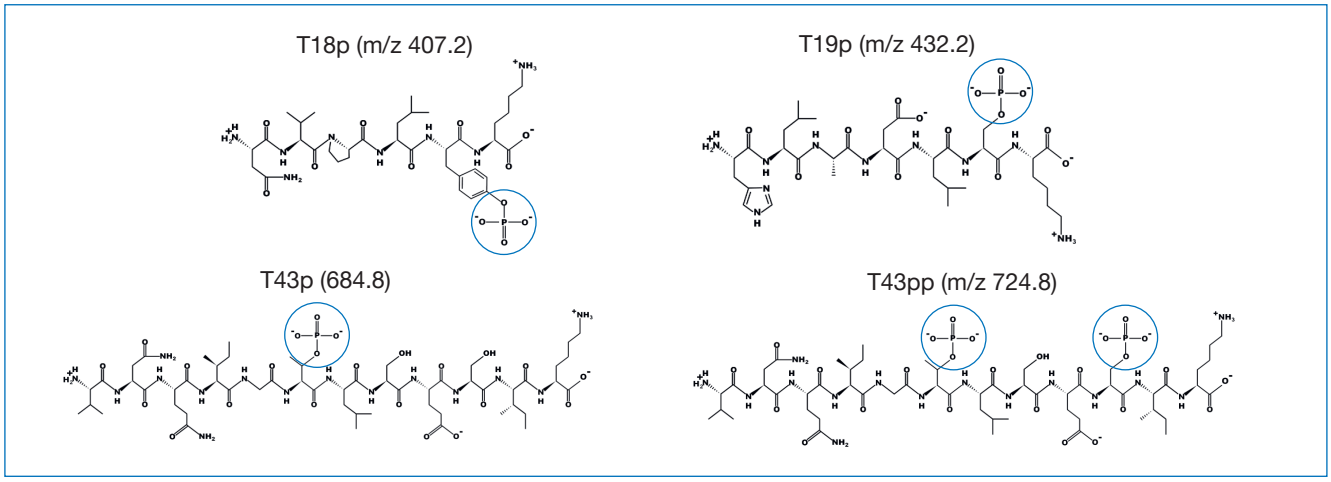

Figure 2: Synthetic phosphorylated peptides used in this application.

Figure 3 shows that use of the bioinert YMC-Accura Triart C18 column led to higher intensities and peak areas for all peaks. Additionally, the high recovery rate from the YMC-Accura Triart C18 column also enabled the detection of the challenging phosphopeptide T43pp, which contains two phosphate residues. In contrast, the analysis with the standard column hardware showed no signal, even after thorough equilibration after several sample injections.

Table 1: Chromatographic conditions.

Columns:	YMC-Accura Triart C18 (1.9 µm, 12 nm) 100 x 2.1 mm ID (bioinert hardware) YMC-Triart C18 (1.9 µm, 12 nm) 100 x 2.1 mm ID (standard hardware)
Part Nos.:	TA12SP9-10Q1PT TA12SP9-10QTPTC
Eluent:	A) water + 0.1% formic acid B) acetonitrile + 0.1% formic acid
Gradient:	0.7%–25%B (0–5 min), 25%B (5–6.6 min), 0.7%B (6.6–8 min)
Flow rate:	0.6 ml/min
Temperature:	60 °C
Detection:	ESI-MS
Injection:	2 µl (10 pmol/µl)
Sample:	Massprep phosphopeptide enolase standard (Waters)
System:	Shimadzu Nexera XS inert Shimadzu LCMS-2020

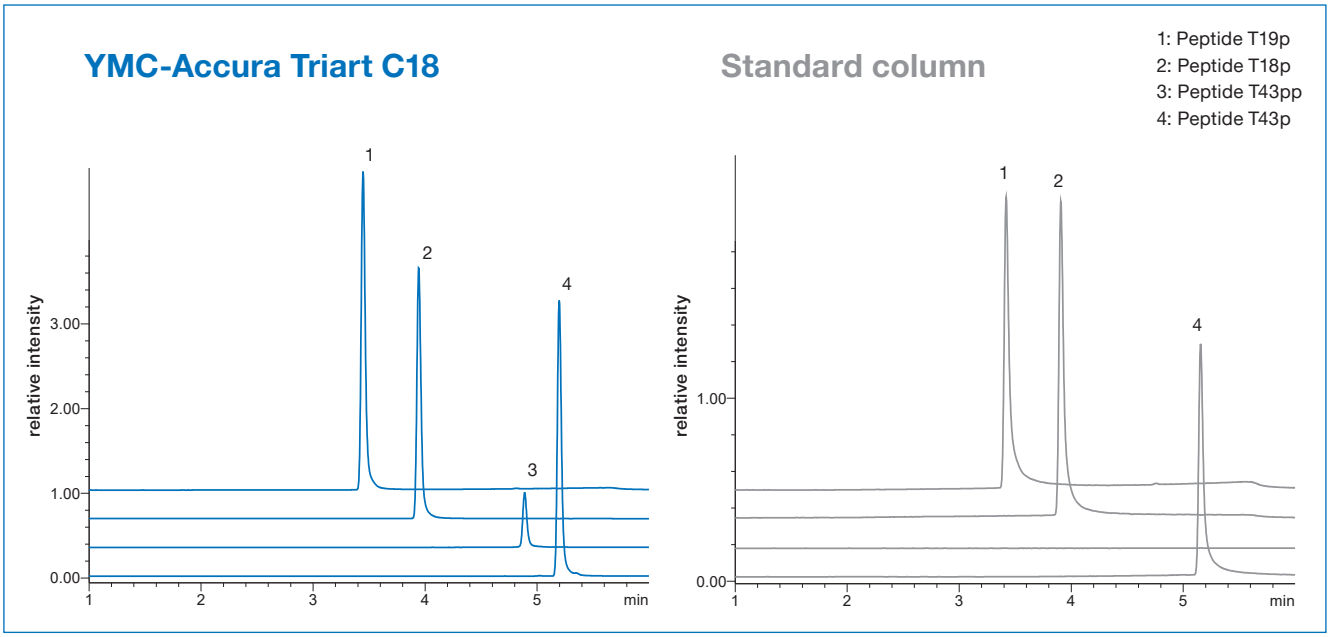

Figure 3: Extracted ion chromatograms (EICs) of phosphopeptide mixture separated with the bioinert YMC-Accura Triart C18 column and the stainless steel column hardware equilibrated with 10 injections.

The bioinert YMC-Accura Triart C18 column provided very stable peak areas. The deviation within the first 10 injections was about 6–9% for T18p, T19p and T43p. Only T43pp saturated after 10 injections with about 32% increase in peak area (figure 4). This proves that little or no column conditioning is necessary with the YMC-Accura Triart C18 column.

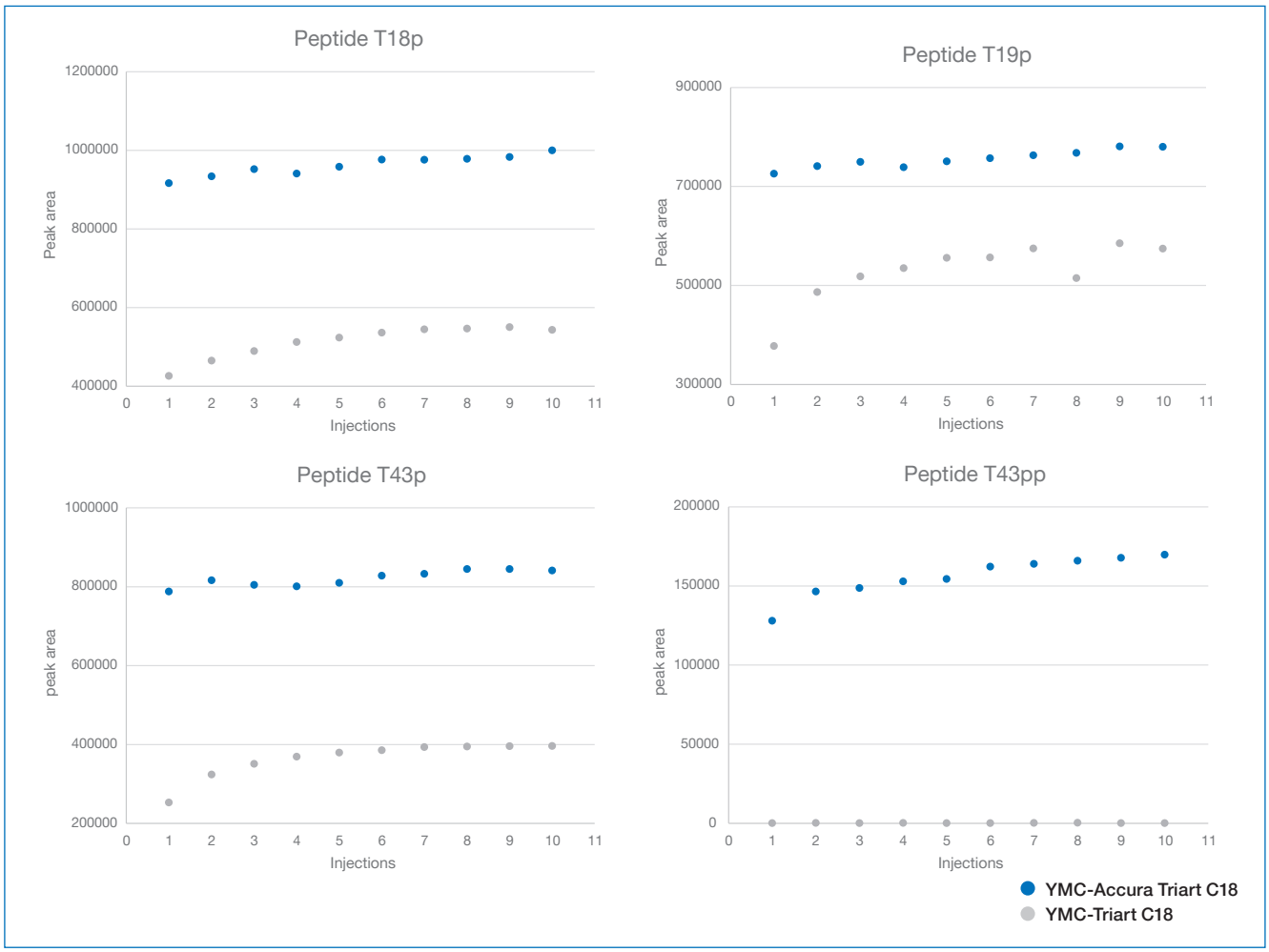

Figure 4: Peak areas of the four phosphorylated peptides after 1-10 injections using the bioinert YMC-Accura Triart C18 column (blue) and the stainless steel standard column (grey).

YMC-Accura Triart C18 column showed very reproducible peak performance. Almost no peak tailing could be observed (figure 5).

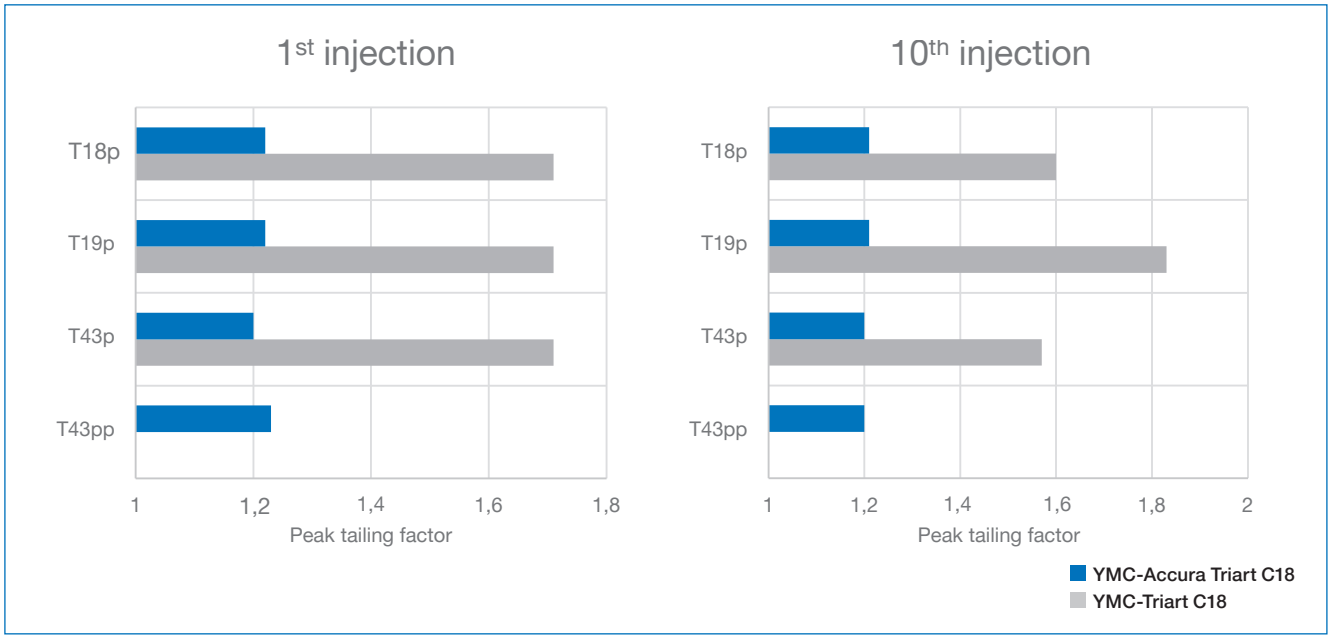

Figure 5: Peak tailing factor of the four phosphopeptides analysed by the bioinert YMC-Accura Triart C18 column (blue) and the stainless steel standard column (grey) after A) the first injection and B) the tenth injection.

These results demonstrate that the bioinert hardware is an essential tool for the analysis of biomolecules. In this case, all peptides were detected with much higher recovery rate than with the standard column hardware. Even challenging peptides can be analysed reproducibly. The use of bioinert YMC-Accura Triart columns has several benefits:

- **Higher recovery**
- **Better peak shapes**
- **Greater reproducibility**
- **Little or no conditioning required**

This makes YMC-Accura Triart columns an excellent choice for the analysis of critical biomolecules such as phosphopeptides.

Application data by courtesy of Shimadzu Europa GmbH.