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Applikationsbericht

Robust Capillary and Low-Microflow LC-MS with Improved Acidic Peptide Recovery Using BioResolve™ 300 µm ID Columns

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Abstract

In this application note, the performance of Waters BioResolve Premier Peptide 300 µm internal diameter (ID) Columns incorporating MaxPeak™ High Performance Surfaces (HPS) Technology is evaluated for LC-MS proteomics analyses. These columns provide superior recovery and improved peak shape for acidic peptides compared to conventional stainless-steel columns while maintaining comparable peak capacity. In addition, excellent column-to-column reproducibility is demonstrated. These results underscore the value of HPS Technology for robust, high-performance capillary and low-microflow LC-MS proteomics workflows, particularly those targeting acidic and phosphorylated peptides.

Benefits

- Higher recovery and improved peak shape for acidic peptides
- Consistent performance from the first injection with minimal column conditioning required
- Robust and reproducible capillary and low-flow separations with excellent column-to-column reproducibility

Introduction

Capillary and microflow LC-MS are increasingly adopted in proteomics to improve sensitivity while maintaining robustness. Columns with a 300 µm ID enable operation at low-microflow and capillary flow rates, which significantly reduces sample and solvent consumption and improves ionization efficiency. These attributes are particularly beneficial for proteomics workflows, where sample amounts are often limited and sensitivity is critical.[1]

Waters 300 µm ID columns deliver high chromatographic efficiency and robust performance for LC-MS-based applications, enabling confident peptide identification and quantitation.[2,3] However, interactions between column hardware and acidic peptides can negatively impact chromatographic performance. These interactions may result in reduced recovery, peak tailing, and increased variability, which can compromise quantitative and qualitative proteomics results.

Waters MaxPeak Premier Columns address these challenges through MaxPeak HPS Technology, which minimizes unwanted analyte interactions with stainless-steel column hardware. HPS Technology has been shown to improve recovery and peak shape for acidic peptides while reducing the need for extensive column conditioning.[4–8] In this application note, the performance of Waters BioResolve Peptide 300 µm ID Columns with MaxPeak Premier Technology in LC-MS proteomics workflows is evaluated.

The BioResolve Peptide MaxPeak Premier 300 µm Columns demonstrate improved recovery and peak shape for acidic peptides compared to equivalent stainless-steel columns, while maintaining comparable peak capacity. Additionally, these columns exhibit excellent column-to-column reproducibility and stable performance over repeated injections. Together, these results highlight the suitability of these columns for routine capillary and low-microflow LC-MS proteomics analyses, including workflows targeting acidic peptide populations.

Experimental

Sample Description

Waters MassPREP™ Enolase Digest with Phosphopeptides Mix (p/n: 186003286 < https://www.waters.com/nextgen/global/shop/standards--reagents/186003286-massprep-enolase-digest-with-

phosphopeptides-mix.html>) was reconstituted in 100 µL of 0.1% formic acid in water.

Calculations

4σ peak capacities were determined based on the following equation:

$$P_{c,4\sigma} = 1 + [(\frac{2.35}{4})(\frac{t_{gradient}}{W_{h,avg}})]$$

LC Conditions

LC system and setup:	ACQUITY™ UPLC™ M-Class System 25 µm ID x 20″ ZenFit tubing (p/n: 700011466) to connect the Micro Sample Manager (µSM) to the Micro Binary Solvent Manager (µBSM), 40 µm ID x 20 in ZenFit tubing (p/n: 430004967) to connect µBSM to column. Flow diverted straight from the column to the MS source with 40 µm ID x 30 in tubing (p/n: 700011516).
Columns:	Waters BioResolve Peptide C_{18} RP Column, MaxPeak Premier Technology, 1.7 µm, 300 Å, BEH™ Column, 0.3 x 50 mm (p/n: 186011501) nanoEase M/Z Peptide BEH C_{18} Column, 300 Å, 1.7 µm, 300 µm x 50 mm (p/n: 186009263)
Column temperature:	60 °C
Sample temperature:	6 °C
Injection volume:	0.5 µL

Mobile phase A:	0.1% Formic Acid in H_2O
Mobile phase B:	0.1% Formic Acid in ACN
Sample vials:	QuanRecovery™ MaxPeak 12 x 32 mm Propylene 300 µL Screw Cap Vials (p/n:186009186)

Xevo G3 Detector Settings

ESI probe:	Low-flow probe (p/n: 186007529)
Mass range:	50–2000 *m/z*
Mode:	ESI+
Sample rate:	2 Hz
Cone voltage:	30 V
Source temperature:	120 °C
Desolvation temperature:	250 °C
Capillary voltage:	2.50 kV
Cone gas:	50 L/h
Desolvation gas:	350 L/h
Lockmass:	LeuEnk (556.27658 *m/z*)
Informatics:	MassLynx Software (data acquisition) and waters_connect™ Software (data processing)

Gradient Table

Time (min)	Flow Rate (mL/min)	%A	%B	Curve
Initial	13.5	99	1	Initial
36	13.5	60	40	6
36.5	13.5	15	85	6
37.4	13.5	15	85	6
38.3	13.5	99	1	6
43.3	13.5	99	1	6

Peptide Sequences

Peptide	Sequence
T11	NVNDVIAPAFVK
T18	NVPLYK
T18p	NVPLpYK
T19p	HLADLpSK
T23	IGSEVYHNLK
T50	LNQLLR
T51	IEEELGDNAVFAGENFHHGDK

Results and Discussion

Figure 1 shows the total ion chromatograms (TICs) of the MassPREP Enolase Digest with Phosphopeptides Mix on a BioResolve C_{18} RP Column and a stainless-steel nanoEase Peptide C_{18} Column. The first injection is shown in blue (top) and orange (bottom), and the tenth injection on each column is shown in black. On the BioResolve C_{18} RP Column, the first and tenth injections are comparable, demonstrating that minimal column conditioning is required when using MaxPeak Premier hardware. In contrast, the nanoEase Peptide C_{18} stainless-steel column requires substantial conditioning. Peaks marked with an asterisk show a pronounced increase in intensity from the first to the tenth injection, indicating non-specific analyte-hardware interactions during the initial injection. Column conditioning reduces these interactions, resulting in increased peak intensities by the tenth injection. The inset highlights a region of significant change on the stainless-steel column between the first and tenth injection; the same region shows little change between the first and tenth injections on the MaxPeak Premier Column. Notably, on the tenth injection, both columns display comparable peak profiles and equivalent peak capacities.

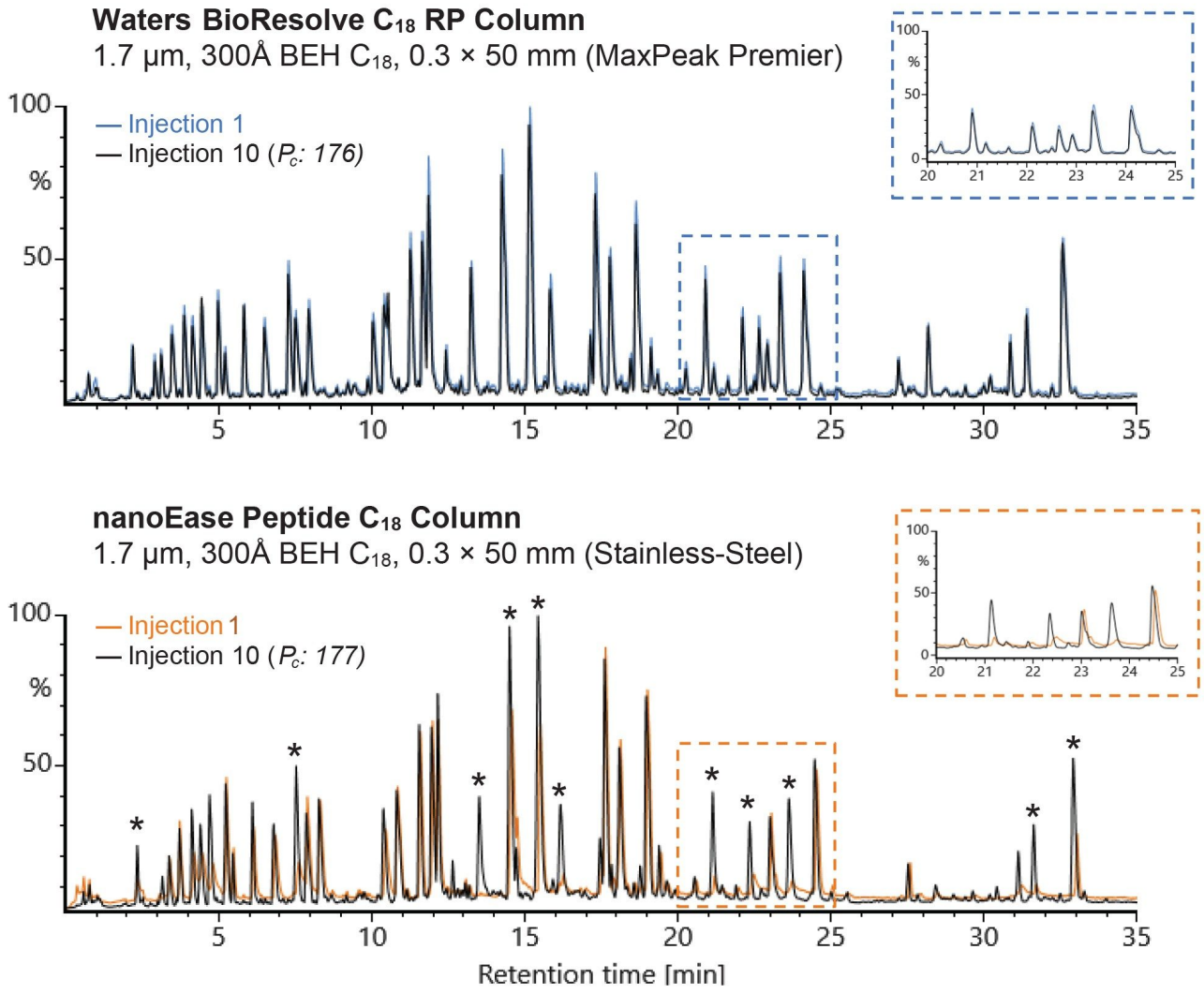

Figure 1. TICs of the MassPREP Enolase Digest with phosphopeptides mix on a Waters BioResolve Peptide C_{18} RP Column, MaxPeak Premier Technology, 1.7 µm, 300 Å BEH, 0.3 x 50 mm Column (top) and a nanoEase M/Z Peptide BEH C_{18} 300 Å, 1.7 µm, 0.3 x 50 mm Column (bottom). The first injection is shown in blue (top) and orange (bottom), and the tenth injection on each column is shown in black. Peak capacities calculated from the tenth injection on each column are listed in the legend. Peaks denoted with an asterisk exhibit a pronounced increase in intensity between the first and tenth injections. The inset highlights a region of significant change on the stainless-steel column between the first and tenth injection; the same region shows little change between the first and tenth injection on the MaxPeak Premier Column.

HPS hardware mitigates unwanted interaction between acidic analytes and column hardware, resulting in higher recovery and improved peak shape relative to stainless-steel. Figure 2 shows the extracted ion chromatograms (XICs) of two acidic peptides (T19p and T51) and a non-acidic reference peptide (T18) analyzed on a Waters BioResolve C_{18} RP Column (left) and a nanoEase Peptide C_{18} Column (right). The T51 peptide contains three consecutive glutamic acid residues, an additional glutamic acid residue, and two aspartic acid residues, while T19p contains one aspartic acid residue and a phosphorylated serine residue. Data from the first and tenth injection on each column are shown. Visual inspection reveals superior recovery of the T51 peptide and improved peak shape for the T19p peptide on the BioResolve C_{18} RP Column during the first injection. In contrast, on the first injection of the nanoEase Peptide C_{18} Column, recovery of the T51 peptide is minimal and the T19p peptide exhibits pronounced peak tailing.

The plots on the right of Figure 2 summarize the relative peak area of the acidic T51 peptide and the USP tailing factor of the phosphorylated T19p peptide as a function of injection number for both columns. The BioResolve C_{18} RP Column demonstrates high T51 recovery on the first injection, with a modest increase in relative peak area over the first three injections before stabilizing by the fourth injection. In contrast, the stainless-steel nanoEase Peptide C_{18} Column exhibits very low T51 recovery on the first injection. Although recovery improves with conditioning, comparable response levels to the BioResolve C_{18} RP Column are not achieved until the tenth injection. Similar trends are observed for the peak shape of the T19p phosphopeptide. On the first injection, T19p exhibits significantly greater tailing on the stainless-steel nanoEase Peptide C_{18} Column compared to the BioResolve Peptide C_{18} RP Column, with comparable tailing factors not observed until the fifth injection. Collectively, these results highlight the improved recovery and peak shape afforded by HPS technology for capillary and low-microflow separations of acidic peptides.

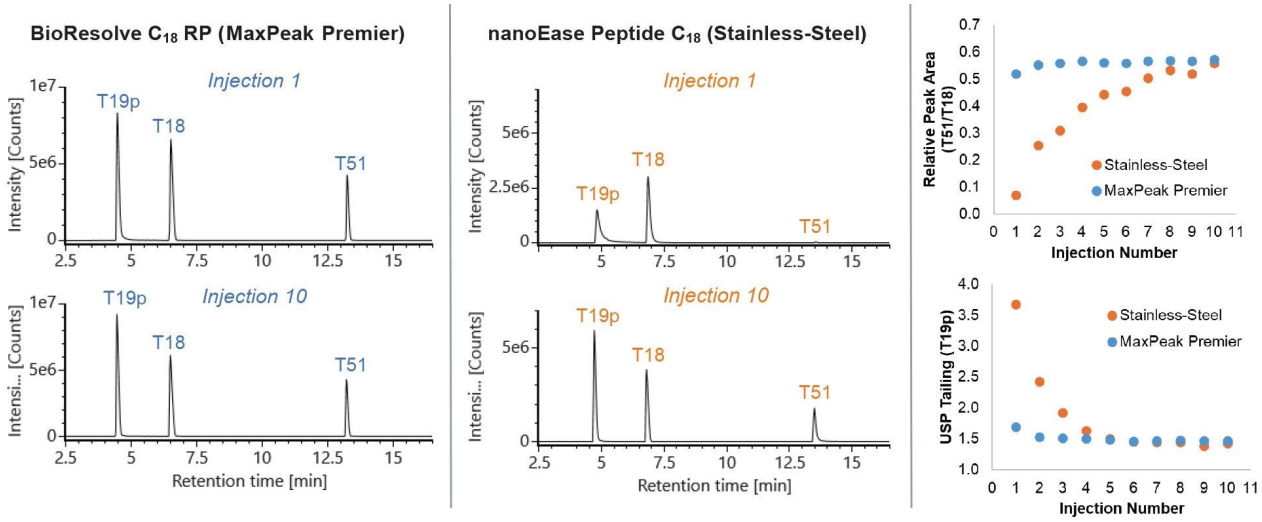

Figure 2. XICs of two acidic peptides (T19p and T51) and a non-acidic reference peptide (T18) from the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve Peptide C_{18} RP Column, MaxPeak Premier 1.7 µm 300 Å BEH 0.3 x 50 Column mm (left) and a nanoEase Peptide BEH C_{18} 300 Å, 1.7 µm, 1.0 x 50 mm Column (middle). Data from the first and tenth injections on each column are shown. Sequences for each peptide are listed in the experimental section. The relative peak area of the T51 peptide and the USP tailing factor of the T19p peptide are plotted as a function of injection number on the right.

Figure 3 displays TICs of the MassPREP Enolase Digest with Phosphopeptides Mix on three BioResolve C_{18} RP 300 µm ID Columns. Excellent reproducibility is observed across the three columns. Using XIC data, relative peak areas and relative retention times were calculated against the T18 peptide. Relative retention time RSDs are less than 1% for each peptide and relative peak area RSDs are less than 5% for each peptide.

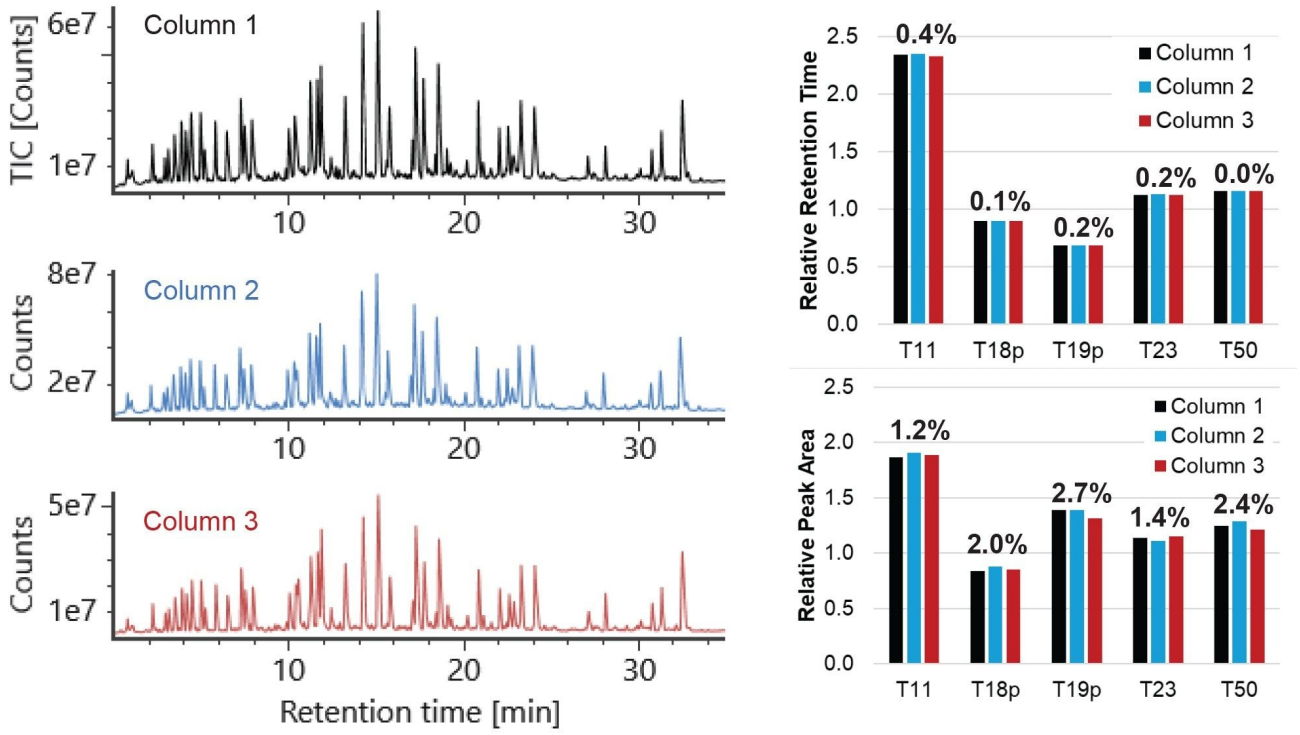

Figure 3. TICs of the MassPREP Enolase Digest with Phosphopeptides Mix on three Waters BioResolve C_{18} RP Column, MaxPeak Premier 1.7 µm 300 Å BEH 0.3 x 50 mm Column (left) and bar charts representing the relative peak areas and relative retention times for selected peptides on each column (right). RSDs are shown above each set of data. Sequences for each peptide are listed in the experimental section.

Conclusion

The results presented in this application note demonstrate that Waters BioResolve Peptide 300 µm ID Columns with MaxPeak Premier Technology deliver clear performance advantages over unmodified stainless-steel columns for capillary and low-microflow LC-MS proteomics analyses involving acidic and phosphopeptides. By leveraging MaxPeak HPS Technology, these columns effectively mitigate deleterious interactions between acidic peptides and column hardware, leading to improved recovery, superior peak shape, and reduced reliance on extensive column conditioning. Importantly, these benefits are achieved while maintaining excellent peak

capacity coupled with improved signal intensity and column-to-column reproducibility. Collectively, these attributes make BioResolve Peptide 300 µm ID Columns a robust and reliable solution for routine proteomics workflows, particularly those focused on acidic peptides where sensitivity, reproducibility, and data quality are paramount.

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