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アプリケーションノート

Enhanced Recovery and Peak Shape of Acidic Peptides with BioResolve™ 1 mm ID Columns with MaxPeak™ Premier Technology

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Published on February 16, 2026

Abstract

Microflow chromatography is increasingly favored in proteomics for its enhanced sensitivity and reduced solvent consumption. However, the recovery and peak shape of acidic peptides remain challenging due to non-specific adsorption to stainless steel column hardware. This application note evaluates the performance of Waters BioResolve 1 mm ID Columns with MaxPeak Premier Technology, which incorporate MaxPeak High Performance Surfaces (HPS) Technology to mitigate unwanted interactions between acidic analytes and column hardware. Using the MassPREP™ Enolase Digest with Phosphopeptides Mix, the BioResolve BEH™ C_{18} RP MaxPeak Premier 1 mm ID Column was compared to a conventional stainless steel ACQUITY™ Peptide BEH C_{18} Column. The MaxPeak Premier Column demonstrates superior recovery and peak shape for acidic peptides from the first injection, eliminating the need for extensive column conditioning. Additionally, it maintains comparable resolution and peak capacity, exhibits excellent column-to-column reproducibility, and is compatible with both microflow and analytical UHPLC systems.

Benefits

- Improved recovery and peak shape of acidic analytes including phosphopeptides while maintaining resolution and peak capacity
- Excellent column-to-column reproducibility
- Versatile compatibility with microflow and analytical UHPLC systems

Introduction

Microflow chromatography offers several advantages over traditional analytical scale chromatography in proteomics applications. Microbore columns require less sample load and enable the use of low flow rates (10–100 µL/min). These low flow rates decrease solvent consumption and optimize the interface with MS, increasing MS sensitivity. Waters microbore columns with a 1 mm internal diameter (ID) demonstrate robust separation capabilities and enable sensitive MS detection in proteomics workflows.[1,2] However, recovery and peak shape of acidic peptides using microbore columns remains a challenge due to non-specific adsorption to the column hardware.

Waters MaxPeak Premier Columns use MaxPeak HPS Technology to mitigate unwanted interactions between acidic analytes and column hardware. In analytical-scale chromatography, HPS Technology improves recovery of acidic peptides, including phosphopeptides, and reduces the amount of column conditioning required to achieve reproducible chromatography.[3,4] This application note describes the benefits of HPS Technology in microflow chromatography using Waters BioResolve Peptide 1 mm ID Columns with MaxPeak Premier Technology. The BioResolve Premier Peptide 1 mm Columns yield improved recovery and peak shape of acidic peptides relative to the stainless steel equivalent while maintaining comparable resolution and peak capacity. Moreover, these columns exhibit excellent column-to-column reproducibility and can be used on both microflow and standard analytical UHPLC systems.

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:

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

LC Conditions

LC system and setup:	ACQUITY UPLC™ M-Class System: 75 µm ID x 30” ZenFit™ tubing (p/n: 700011513) to connect the micro sample manager (µSM) to the 20 µL mixer (p/n: 289003345), 75 µm ID x 14” ZenFit tubing (p/n: 700011506) to connect the mixer to the micro binary solvent manager (µBSM), 75 µm ID x 26 in ZenFit tubing (p/n: 700011505) 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). ACQUITY Premier UPLC System: Standard tubing prior to column, 0025” tubing to connect column to the MS divert valve.
Columns:	Waters BioResolve Peptide C_{18} RP Column, MaxPeak Premier Technology, 1.7 µm, 300 Å, BEH, 1.0 x 50 mm (p/n: 186011513) ACQUITY Peptide BEH C_{18} 300 Å Column, 1.7 µm, 1.0 x 50 mm (p/n: 186005592)
Column temperature:	60 °C
Sample temperature:	6 °C

Injection volume:	1 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 (M-class) and waters_connect™ Software (ACQUITY Premier

UPLC)

Gradient Table

Time (min)	Flow (µL/min)	%A	%B	Curve
Initial	50	99	1	Initial
18	50	60	40	6
18.5	50	15	85	6
19.4	50	15	85	6
20.3	50	99	1	6
25.3	50	99	1	6

Results and Discussion

Figure 1 shows the total ion chromatograms (TICs) of the MassPREP Enolase Digest with Phosphopeptides Mix on a BioResolve Peptide C_{18} RP Column and a stainless steel ACQUITY Peptide BEH C_{18} Column. The two columns produce similar peak profiles with slight differences in resolution for certain peptides (see peaks denoted with an asterisk). The resolution differences observed are likely a result of differences in selectivity between the two columns. The peak capacity of the MaxPeak Premier Column is comparable to that of the stainless steel column.

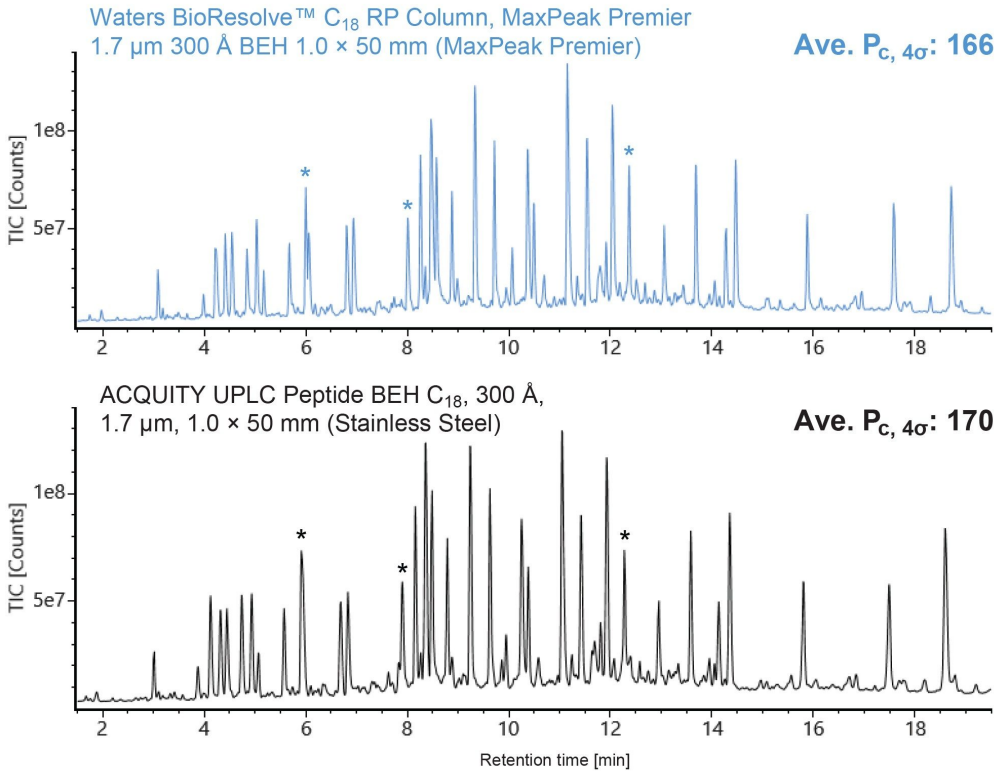

Figure 1. Total ion chromatograms of the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve Peptide C_{18} RP Column, MaxPeak Premier Technology, 1.7 µm, 300 Å BEH, 1.0 x 50 mm (blue trace) and an ACQUITY Peptide BEH C_{18} 300 Å, 1.7 µm, 1.0 x 50 mm Column (black trace). Both columns were run on the M-class system. The MaxPeak Premier HPS hardware delivers comparable resolution and peak capacity to the stainless steel hardware.

HPS hardware mitigates unwanted interaction between acidic analytes and column hardware, resulting in high recovery and improved peak shape relative to stainless steel. Extracted ion chromatograms (XICs) for two acidic peptides on a BioResolve Peptide C_{18} RP Column and a stainless steel ACQUITY Peptide BEH C18 Column are shown in Figure 2. The T51 peptide possesses three glutamic acid residues in series plus an additional glutamic acid residue and two aspartic acid residues. The second peptide, T19p, is a phosphopeptide. Data is shown for the first and 10th injections on each column. The BioResolve C_{18} RP Column exhibits high recovery of the T51 peptide on the first injection; consistent with that observed for injection 10. On the stainless steel ACQUITY Peptide BEH C18 Column, low recovery of the T51 peptide is observed on the first injection. With conditioning, recovery increases to levels comparable to the BioResolve Premier Column by the 10th injection.

Similar results are observed for peak shape of the T19p phosphopeptide. On the first injection, the T19p peptide exhibits significantly higher tailing on the stainless steel ACQUITY Peptide BEH C_{18} Column compared to the BioResolve Peptide C_{18} RP Column. Symmetrical peaks are observed by the 10th injection on both columns. These results highlight the improved recovery and peak shape benefits of HPS technology through microflow separations of acidic peptides.

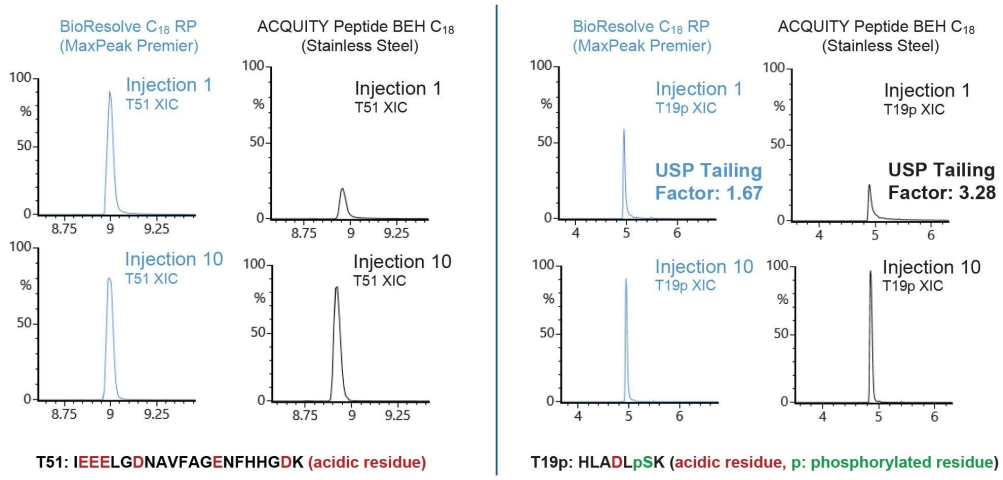

Figure 2. Extracted ion chromatograms of two acidic peptides from the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve™ C_{18} RP Column, MaxPeak Premier 1.7 µm 300 Å BEH 1.0 x 50 mm (blue traces) and an ACQUITY Peptide BEH C_{18} 300 Å, 1.7 µm, 1.0 x 50 mm Column (black traces). Both columns were run on the ACQUITY UPLC M-Class System.

Figure 3 displays overlaid TICs of the MassPREP Enolase Digest with Phosphopeptides Mix on three BioResolve C_{18} RP 1 mm 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 10% for each peptide.

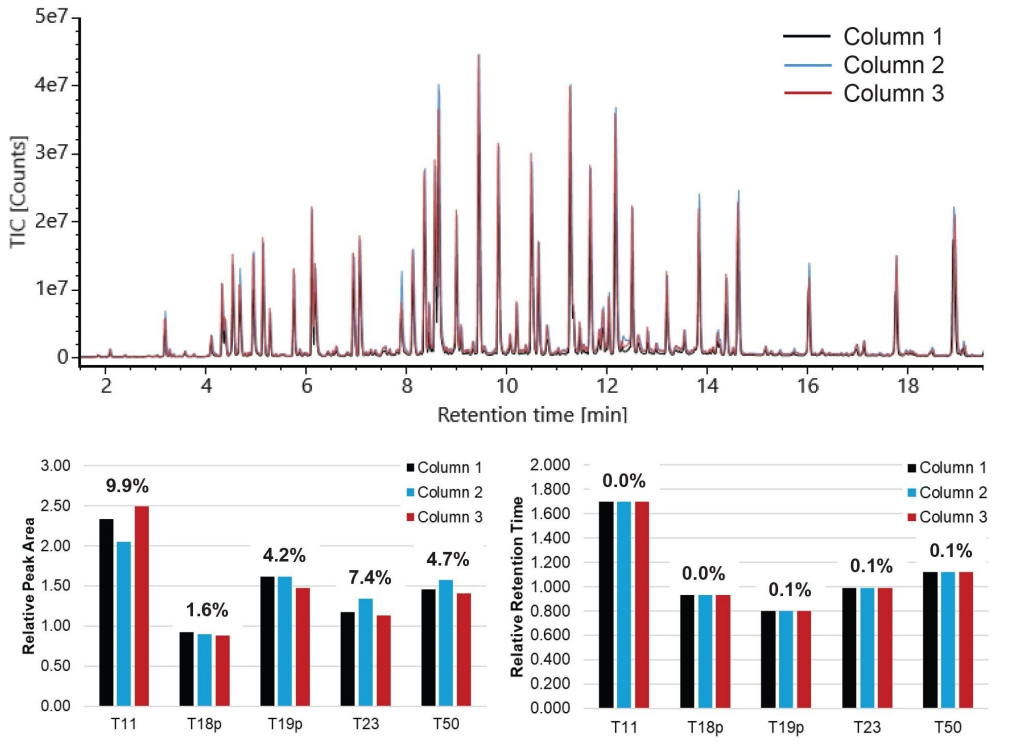

Figure 3. Overlaid total ion chromatograms of the MassPREP Enolase Digest with Phosphopeptides Mix on three Waters BioResolve C_{18} RP Columns, MaxPeak Premier 1.7 µm 300 Å BEH 1.0 x 50 mm (top) and bar charts representing the relative peak areas and relative retention times for selected peptides on each column (bottom). RSDs are shown above each set of data. All columns were run on the ACQUITY UPLC M-Class System.

All data presented above were acquired using an ACQUITY UPLC M-Class System. Waters Premier 1 mm Columns can be used on both microflow and standard analytical flow UHPLC instruments. Figure 4 displays the TIC of the MassPREP Enolase Digest with Phosphopeptides Mix on a 1.0 x 50 mm BioResolve C_{18} RP Column using an ACQUITY Premier UPLC System. Comparable peak profiles are obtained using both the ACQUITY UPLC M-Class System and ACQUITY Premier UPLC System. As expected, peak capacity for the BioResolve C_{18} RP 1.0 x 50 mm Column decreases on the ACQUITY Premier UPLC System due to increased impact of post-column dispersion but is comparable to Premier 2.1 x 50 mm Columns run on the same system (Figure 4, right).

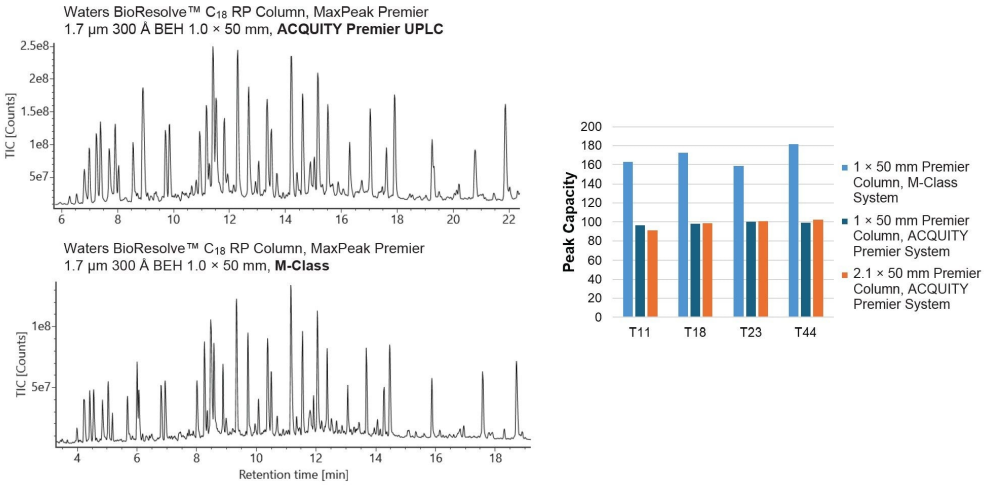

Figure 4. Total ion chromatograms of the MassPREP Enolase Digest with Phosphopeptides Mix on a Waters BioResolve C_{18} RP Column, MaxPeak Premier 1.7 µm 300 Å BEH 1.0 x 50 mm on the ACQUITY Premier UPLC System (top) and ACQUITY UPLC M-Class System (bottom). Peak capacities for selected peptides are plotted on the right. Peak capacity data is shown for the BioResolve Peptide RP C_{18}, MaxPeak Premier Technology, 1.7 µm, 300 Å, BEH, 1.0 x 50 mm column on the ACQUITY UPLC M-Class System and ACQUITY Premier System and the ACQUITY Premier Peptide BEH C_{18}, 300 Å, 1.7 µm, 2.1 x 50 mm Column on the ACQUITY Premier System.

Conclusion

Waters BioResolve 1 mm ID Columns equipped with MaxPeak Premier Technology significantly enhance the chromatographic separation of acidic and phosphopeptides in microflow LC-MS workflows. Compared to stainless steel hardware, these columns deliver improved peptide recovery and peak shape from the first injection, reducing the need for conditioning. The MaxPeak Premier Columns maintain high resolution and peak capacity, demonstrate excellent reproducibility across columns, and are versatile across both microflow and analytical UHPLC platforms. These attributes make them a robust and reliable choice for proteomics applications requiring sensitive and reproducible analysis of acidic peptides. Additionally, these columns provide a significant solvent savings option compared to 2.1 mm ID analytical columns, lowering operating costs and contributing to greater ecological sustainability.

References

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720009231, February 2026