Waters™

응용 자료

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Abstract

Identifying improvement opportunities in pharmaceutical product lifecycle management is recommended as a means to improve drug safety and continually expand the body of knowledge in the manufacturing of drug products. In this study we demonstrate the successful migration of a peptide mapping method from a legacy system (ACQUITY UPLC “Classic” System) to the ACQUITY Premier System (fixed-loop design) featuring MaxPeak™ High Performance Surfaces (HPS). Reduction of analyte/surface adsorption artifacts resulted in less peak tailing and improved recovery of critical quality attributes for better lifecycle management of biotherapeutic drug products.

Benefits

The ACQUITY Premier System fixed-loop design offers:

- Consistent and reliable performance with reproducible results
- Seamless method migration from legacy ACQUITY UPLC “Classic” Systems
- MaxPeak HPS Technology for reduced peak tailing and increased recovery of metal-sensitive analytes

Introduction

Lifecycle management plays a critical role in the manufacturing of drug products.

As part of this process, ICH guidance recommends identifying and evaluating opportunities that improve drug safety in a feedback/feedforward fashion to continually expand the body of knowledge in the manufacturing of drug products.[1] This process can be applied to analytical methods and instruments as they become antiquated. With recent technology providing faster, more robust, or more sensitive analytical capabilities, both manufacturers and consumers benefit from the higher confidence and efficiency of results. Recently, Waters™ introduced the ACQUITY Premier System featuring MaxPeak HPS which further improves upon the chromatographic performance established by Waters' LC portfolio. MaxPeak HPS are engineered to reduce analyte/surface interactions that result in adsorption artifacts such as peak tailing and reduced analyte recovery. As part of this offering, the ACQUITY Premier System is now offered in a fixed-loop design allowing scientists to take full advantage of this novel surface technology across their product pipelines.

The goal of this study is to demonstrate that the ACQUITY Premier System with a fixed-loop design can successfully migrate a peptide mapping method from a legacy system such as the ACQUITY UPLC "Classic" System while delivering additional benefit in terms of chromatographic performance with MaxPeak HPS Technology.

Experimental

MS-grade water and acetonitrile were purchased from Honeywell Burdick and Jackson. MS-grade formic acid was purchased from Thermo Scientific™. Samples were prepared using 0.1% formic acid in water at a concentration of 0.8 mg/mL using mAb Tryptic Digestion Standard (p/n: 186009126 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>) purchased from Waters Corporation.

LC Conditions

LC system(s):	ACQUITY Premier System (BSM-FL variant) ACQUITY UPLC System
Detection:	ACQUITY TUV, FC=Ti 5 mm, λ=214 nm

Vials:	QuantRecovery™ MaxPeak Vials, (p/n: 186009186)
Column(s):	ACQUITY UPLC CSH™ C_{18} Column, 130 Å, 1.7 µm, 2.1 x 100 mm, (p/n: 186005297) ACQUITY Premier Peptide CSH C_{18} Column, 130 Å, 1.7 µm, 2.1 x 100 mm (p/n: 186009488)
Column temperature:	60 °C
Sample temperature:	10 °C
Injection mode:	PLUNO
Injection loop:	10 µL
Injection volume:	5 µL
Weak wash volume:	600 µL
Strong wash volume:	200 µL
Flow rate:	0.200 mL/min
Mobile phase A:	H_2O, 0.1% formic acid
Mobile phase B:	MeCN, 0.1% formic acid
Weak wash:	50:50 MeCN:H_2O
Strong wash:	50:50 MeCN:H_2O
Seal wash:	20:80 MeCN:H_2O

Gradiant

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.200	99.0	1.0	Initial
1.00	0.200	99.0	1.0	6
51.00	0.200	65.0	35.0	6
57.00	0.200	15.0	85.0	6
61.00	0.200	15.0	85.0	6
67.00	0.200	99.0	1.0	6
80.00	0.200	99.0	1.0	6

MS Conditions

MS system:	ACQUITY QDa™ Mass Detector
Scan mode:	Positive, full scan
Scan range:	350–1250 *m/z*
Sampling rate:	5 Hz
Probe temperature:	600 °C
Capillary voltage:	1.5 kV
Cone voltage:	15 V

Data Management

Chromatography software:	Empower™ 3, FR4

Results and Discussion

For this method migration study, we chose peptide mapping of a monoclonal antibody digest as a representative method to challenge the system to deliver accurate gradient composition at slower flow rates for complex samples. In addition to being a challenging separation, peptide mapping was chosen given the ubiquity peptide analyses have in the characterization and quality control of protein-based therapeutics. In this respect, peptide mapping serves as a surrogate method to demonstrate the ACQUITY Premier's ability to be broadly deployed across a product's pipeline. Lastly, as the primary building blocks of protein-based therapeutics, acidic amino acids inherently benefit from MaxPeak HPS. Acidic residues that naturally occur in proteins such as glutamic acid or aspartic acid can exhibit a negative charge that can result in peak tailing due to adsorption of peptides to metal surfaces as shown in Figure 1. For biotherapeutics, this can be critically important as deamidation of asparagine to aspartic acid and iso-aspartic acid is a common post-translational modification of monoclonal antibodies (mAbs) that has been correlated to drug efficacy. Given this, it is not only important to demonstrate method equivalency in this case study, but to demonstrate the value MaxPeak HPS Technology offers in these analyses to reduce undesirable adsorption artifacts for better lifecycle management of drug products.

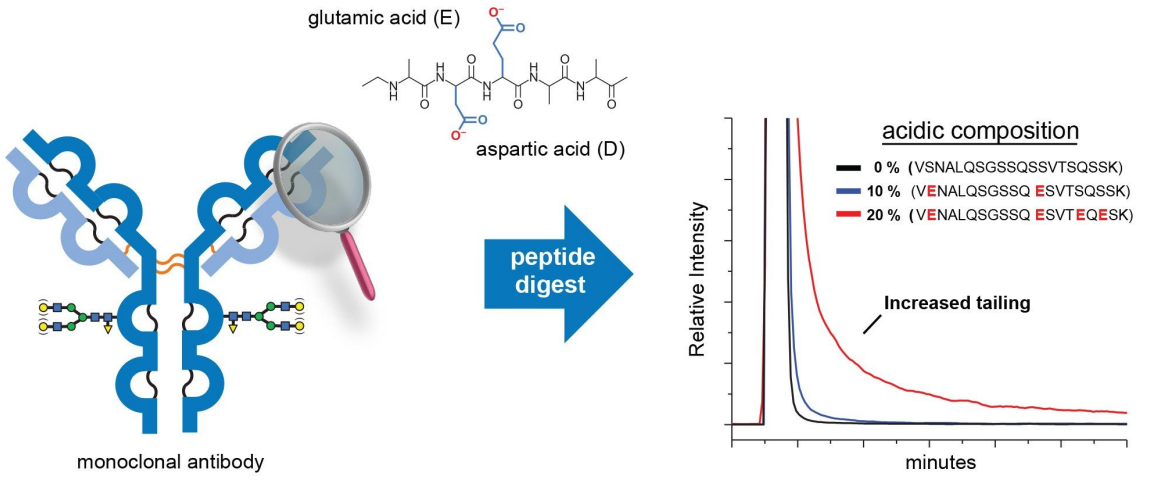

Figure 1. Adsorption artifacts brought on by acidic amino acid residues in proteins such as glutamic and aspartic acid can manifest as peak tailing in peptide mapping profiles.

In the case of the latter, the ACQUITY Premier System with a fixed-loop sample manager was purposefully

engineered to have its needle design and system dwell volume closely match its predecessor, the ACQUITY UPLC "Classic" System, to facilitate easier migration of legacy methods. This is demonstrated in Figure 2, where a simple 25-minute gradient from 5–95% mobile phase B was performed using caffeine to determine dwell volume for both systems.[2] Using the difference between the detector response and the programmed gradient as illustrated in the figure inset, the ACQUITY Premier Systems dwell volume (158.8 µL) was determined to be within 10 µL of the ACQUITY UPLC Systems dwell volume (149.4 µL). This near equivalency means little to no gradient changes are required when migrating methods between systems to achieve the same separation. To highlight this benefit, Figure 3 shows a peptide map of a mAb tryptic digestion standard run on both systems using the same column and method. As shown in the figure, the peptide profiles closely agree in terms of peak retention time and relative intensity for 22 peptide peaks selected throughout the chromatogram with no modification to the method.

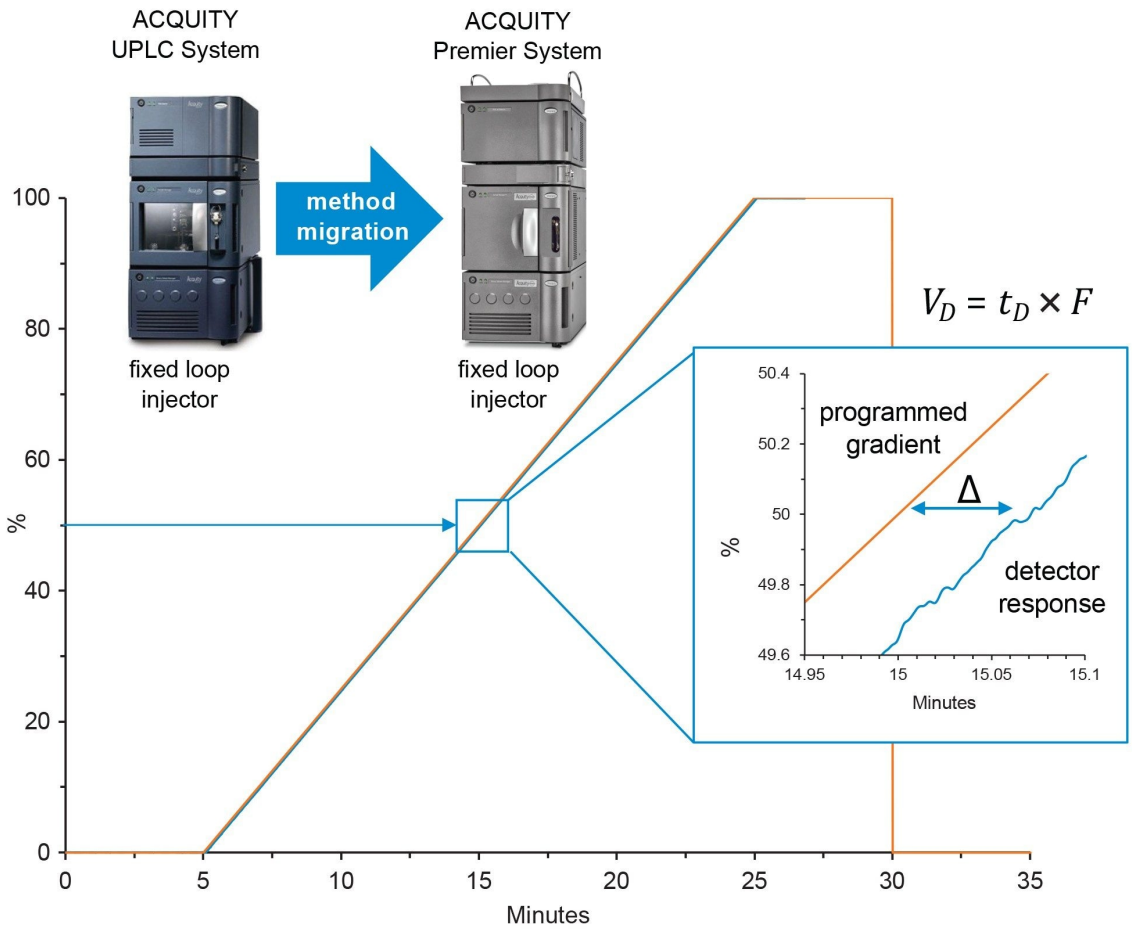

Figure 2. Using caffeine as a tracer molecule to monitor detector response versus the programmed gradient, dwell volume was determined to be 158.8 µL and the 149.4 µL for the ACQUITY Premier System and ACQUITY UPLC System, respectively. The closely matched dwell volume between systems did not constitute method changes in the migrated method.

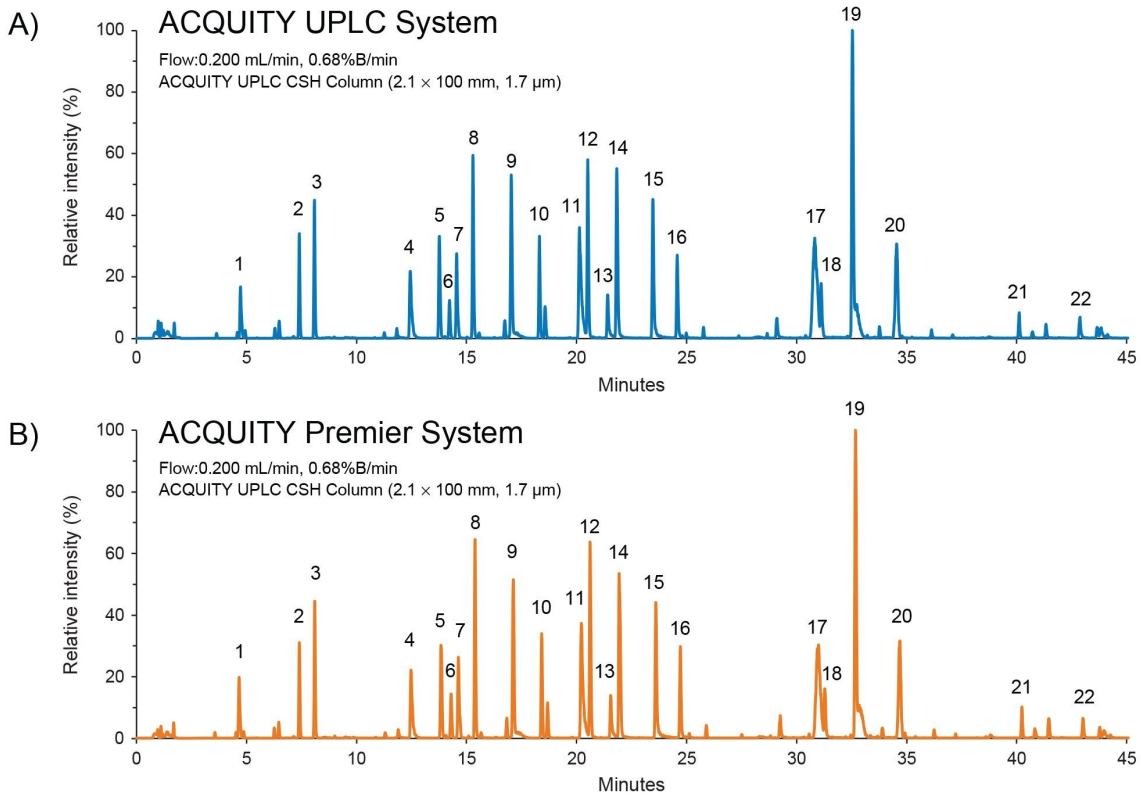

Figure 3. A RPLC separation of Waters mAb Tryptic Digestion Standard using an ACQUITY UPLC CSH Column (2.1 x 100 mm, 1.7 µm) on an A) ACQUITY UPLC System and B) ACQUITY Premier System (fixed-loop design) using the same method and column across both systems.

To evaluate method equivalency, an orthogonal plot of retention time for the 22 peaks shown was made using data from both systems. As shown in Figure 4A, the orthogonal plot exhibited a linear response with a slope of approximately 1.00 and a y-intercept close to zero (0.003) indicating The ACQUITY Premier System was able to reproduce the same selectivity and retention time as the ACQUITY UPLC System. Furthermore, as shown in Figure 4B, peak area was conserved with relative peak area differences being within 0.5% when compared between systems. These results demonstrate that the ACQUITY Premier System can reliably reproduce legacy methods from the ACQUITY UPLC System to facilitate seamless method migration.

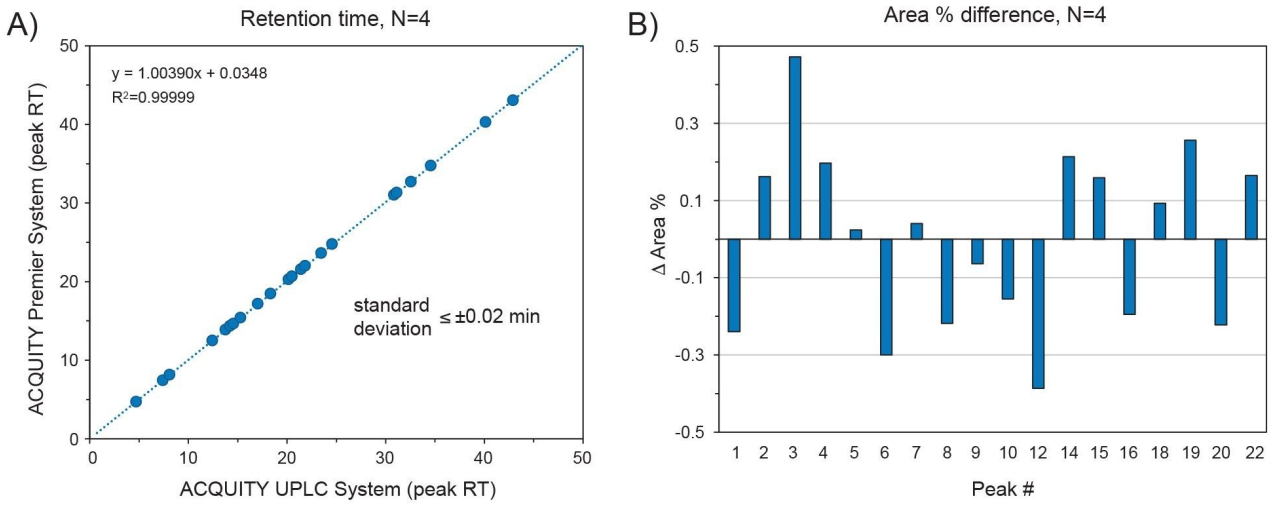

Figure 4. An A) orthogonal plot of absolute retention time and B) relative peak area difference of peptide peaks from a peptide mapping method performed on the ACQUITY UPLC System and ACQUITY Premier System (fixed-loop design) using the same method and column.

With comparability established between systems, the next question was to determine if MaxPeak HPS Technology offers additional benefits to peptide mapping assays. For this experiment, a CSH Column with and without the MaxPeak HPS Technology was used to perform the same peptide mapping analysis on the ACQUITY Premier System. Four peptide peaks exhibited visually noticeable improvements in peak tailing when using the column with MaxPeak HPS Technology as noted in Figure 5. Further investigation revealed that the 4 peptide peaks contained 2–4 "acidic" amino acid residues as indicated by their sequence in the table of Figure 6A. The reduced peak tailing observed in all four peaks allowed for increased recovery in terms of peak area as indicated by the bar plot (Figure 6B). Most notable was the recovery of the LT14 peptide fragment which was below detection thresholds when using the stainless-steel Column. Furthermore, the reduced tailing and higher recovery of acidic peptides provides the ability to see critical quality attribute related impurities. This is demonstrated in Figure 6C for the deamidated species of the critical quality attribute related "penny" peptide, a peptide known to play a role in therapeutic efficacy of mAb-based drug products.[3] In this instance, a 70% reduction in peak tailing of the native peak was observed, enabling detection of the related deamidated impurities. These results demonstrate the value MaxPeak High Performance Surfaces offer in peptide mapping assays and its potential to improve the lifecycle management of biopharmaceuticals.

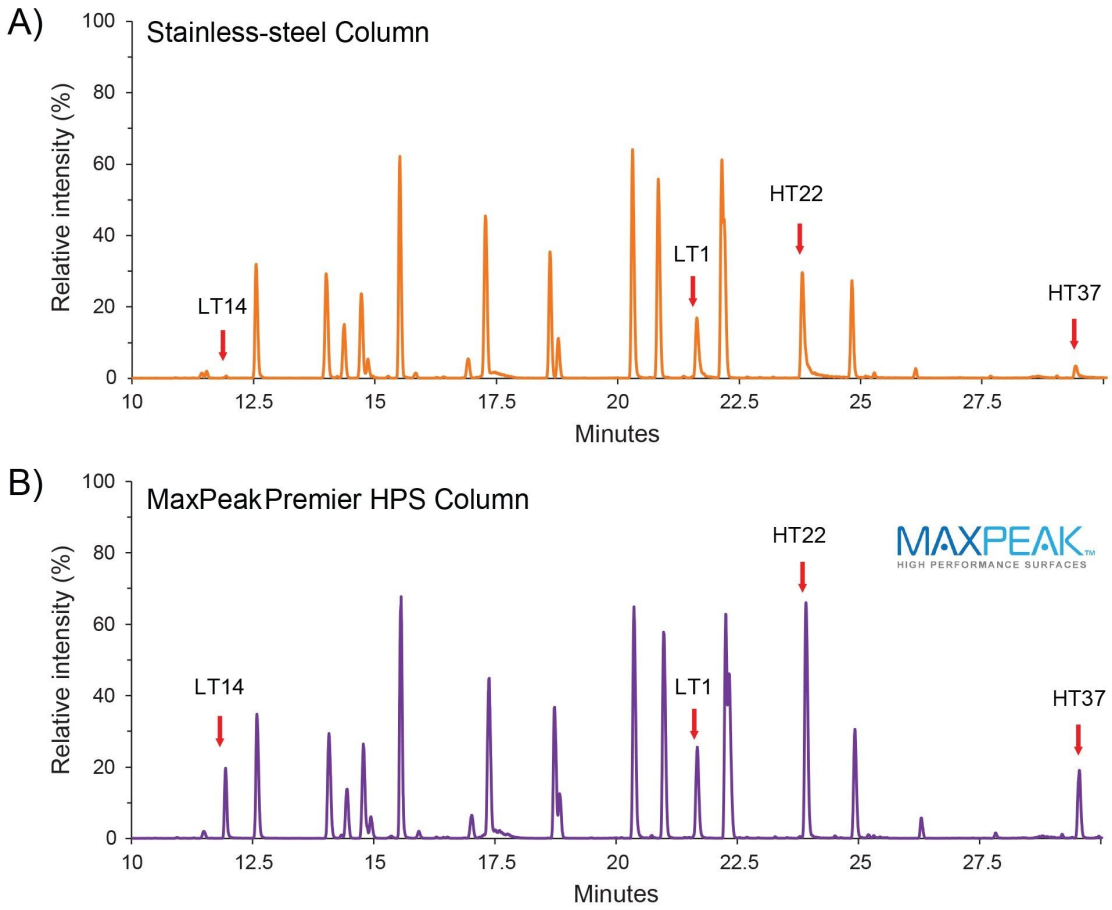

Figure 5. A RPLC separation of Waters mAb Tryptic Digestion Standard on the ACQUITY Premier System using an A) ACQUITY UPLC CSH Column (2.1 x 100 mm, 1.7 µm) and a B) ACQUITY Premier Peptide CSH Column (2.1 x 100 mm, 1.7 µm). Peaks that showed observable peak tailing are noted.

A) "acidic" peptides

Peptide	Sequence	MW (Da)	Tailing factor	
			Stainless steel	MaxPeak HPS
LT14	VDNALQSGNSQESVTEQDSK	2136.2	N.D.	1.3
LT1	DIQMTQSPSTLSASVGDR	1893.1	1.9	1.2
HT22	TPEVTCVVVDVSHEDPEVK	2082.3	2.3	1.3
HT37	GFYPSDIAVEWESNGQPENNYK	2544.7	3.5	1.1

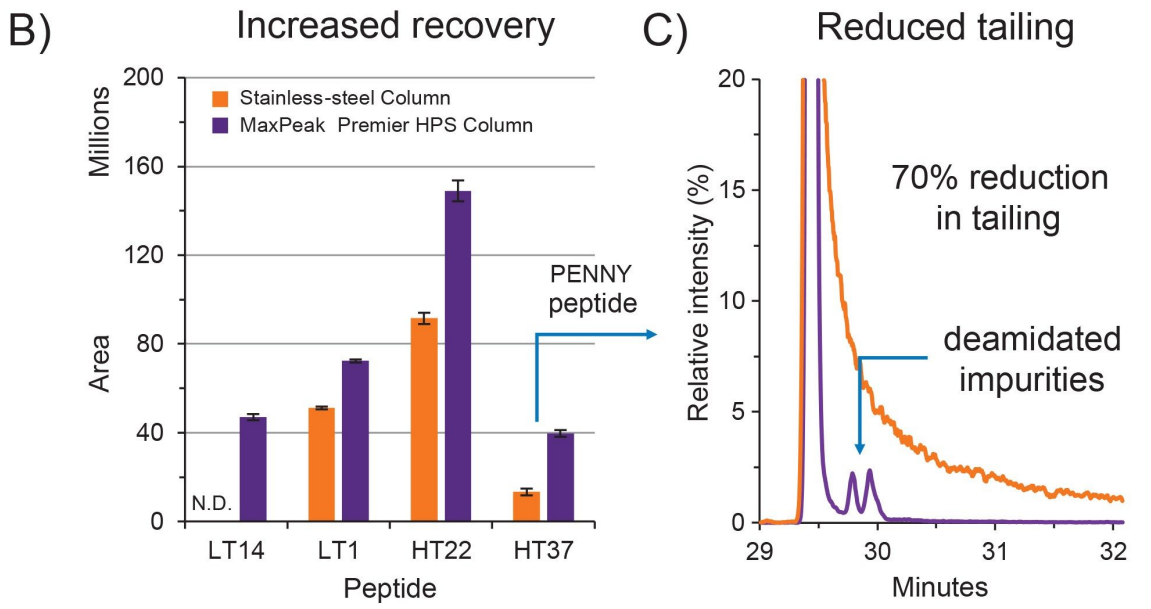

Figure 6. A) Four acidic peptides containing 2-4 acidic amino acids exhibited B) increased recovery and C) up to a 70% reduction in tailing when separated on the ACQUITY Premier System using a ACQUITY Premier Peptide CSH Column (2.1 x 100 mm, 1.7 µm).

Conclusion

Identifying improvement opportunities in pharmaceutical product lifecycle management is recommended as a means to improve drug safety and continually expand the body of knowledge in the manufacturing of drug products. The ACQUITY Premier System featuring MaxPeak HPS represents an innovative leap forward in separation science with its ability to reduce analyte/surface interactions and improve chromatographic performance. With the introduction of the fixed-loop design into the ACQUITY Premier System portfolio, scientists can now take full advantage of this novel surface technology to modernize legacy methods and develop assays that are faster, more robust, and more sensitive for better consumer safety in drug products.

References

1. Guidance for industry Q10 pharmaceutical quality system. ICH, 2009.
2. P. Bigos, R. Birdsall, Y. Yu. Obtaining Equivalent IEX Chromatographic Performance Through Automated Method Scaling Using Waters Column Calculator. Waters Application Note 720007807. 2022.
3. Chelius D, Rehder DS, Bondarenko PV. Identification and Characterization of Deamidation Sites in the Conserved Regions of Human Immunoglobulin Gamma Antibodies. *Anal. Chem.* 77: 6004 2005.

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