Waters™

應用手冊

Improving Peptide Mapping Studies and Reducing Assay Failures Through Reproducible Performance Using the ACQUITY Premier UPLC System (BSM)

Kellen DeLaney, Robert E. Birdsall, Ying Qing Yu

Waters Corporation

Abstract

Reproducibility of liquid chromatography (LC) systems is critical in peptide mapping workflows to simplify sample comparability and ensure accurate peak assignment. In this study, the performance of the ACQUITY™ Premier System, the most recent evolution of the Waters™ ACQUITY UPLC™ bioseparations platform, was investigated. This comprehensive study evaluated the retention time reproducibility across three systems and nine LC separation conditions to evaluate flow rate and gradient impact on assay reproducibility. The results indicate the ACQUITY Premier System is able to deliver consistent retention times with standard deviations of less than 1 second for the majority of peptides investigated under each condition set. Furthermore, the ACQUITY Premier MaxPeak™ High Performance Surfaces (HPS) Technology was shown to substantially improve chromatographic performance by reducing nonspecific peptide adsorption, enabling accurate detection and quantitation of lower-abundance impurities. Overall, these results demonstrate that the ACQUITY Premier System is able to consistently and reproducibly deliver results for peptide mapping workflows that support the needs of development and quality control organizations.

Benefits

- ACQUITY Premier System delivers high inter- and intra-system precision of retention time across a broad set of conditions
- MaxPeak High Performance Surfaces Technology improves chromatographic performance by reducing peak tailing
- Robust performance of the ACQUITY Premier System enables high-confidence attribute analyses and impurity assays

Introduction

The confidence and robustness of peptide mapping in a liquid chromatography (LC) workflow is in part dependent upon the LC pump's ability to deliver the mobile phase gradient composition in a consistent manner. Deviations in composition can impact the retention time and/or peak shape of peptides. In this respect, an LC's pump performance is critical as reproducible retention time and peak shape are necessary to adequately resolve and quantify modifications or critical pairs. Furthermore, ensuring LC performance is reproducible between systems instills confidence that robust methods can be developed and transferred across labs and organizations.

Previous studies have demonstrated the high-performance capabilities of the Waters ACQUITY UPLC Systems for accurately delivering mobile phase compositions across various gradients with highly reproducible retention times of analytes.[1–3] Recently, the ACQUITY Premier UPLC System featuring MaxPeak HPS Technology was introduced as an LC platform that enables greater resolution and sensitivity due to the reduction of nonspecific peptide adsorption.[4–6] The aim of this study is to evaluate the ACQUITY Premier System configured with a Binary Solvent manager (BSM) for delivering high performance peptide mapping assays. Retention time was measured across nine separation conditions using three ACQUITY Premier Systems to assess platform suitability across a broad range of conditions. Peak tailing was used as a metric to evaluate the impact of ACQUITY Premier MaxPeak HPS Technology on chromatographic performance. The results described herein demonstrate how the ACQUITY Premier BSM can easily deliver the high-quality results necessary to support peptide mapping workflows, making it both suitable and flexible for deployment in the development and manufacture of biopharmaceuticals.

Experimental

Sample Description

The sample used for this study was Waters mAb Tryptic Digestion Standard (p/n: 186009126 < https://www.waters.com/nextgen/global/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>) reconstituted in 300 µL mobile phase A (0.133 µg/µL). Mobile phase A was used for blank injections.

LC Conditions

LC System:	ACQUITY Premier UPLC BSM System
Detection:	ACQUITY Premier TUV; 10 mm analytical flow cell; wavelength = 214 nm
Vials:	QuanRecovery with MaxPeak HPS vials (p/n: 186009186)
Column(s):	ACQUITY Premier CSH C_{18} 1.7 µm, 2.1 × 100 mm
Column temp.:	60 °C
Sample temp.:	8 °C
Injection Volume:	10 µL
Flow Rate:	0.200–0.500 mL/min
Mobile Phase A:	0.1% formic acid in water

	(LCMS grade)
Mobile Phase B:	0.1% formic acid in acetonitrile (LCMS grade)
Gradient:	5–95% B; 0.25% –1% B/min

MS Conditions

MS System:	ACQUITY QDa Mass Detector
Ionization Mode:	Positive
Acquisition Range:	350–1250 *m/z*
Capillary Voltage:	1.5 kV
Cone Voltage:	15 V

SIR Values for Retention Time Evaluation

Peptide	*m/z*
LC:T3	406.4
HC:T30	419.9
HC:T21	418.4
LC:T18	626.4
LC:T5	476.9

HC:T11	925.4
HC:T36	581.7
HC:T41	561.5
HC:T23	560.3
LC:T15	752.3
HC:T22	714.2
HC:T37	849.3
HC:T20	712.4
HC:T26	603.8
LC:T11	600.4
HC:T2	926.6
HC:T15	960.5
LC:T7	1122.1

SIR Values for Peak Tailing Evaluation

Peptide	*m/z*
LC:T14	713.1
LC:T3	406.5

LC:T1	477
HC:T22	714.2
HC:T37	849.3
HC:T4-5	597.4
HC:T4	831.3
LC:T7	1122.3

Data Management

Software	Empower3 FR 4

Results and Discussion

In biopharmaceutical assays, reproducible results are critical to instilling confidence in a product and minimizing downtime due to assay deviations warranting investigation. Assays using peptide mapping often require an LC system to deliver shallow gradients at slow flow rates to resolve complex peptide profiles. As such, it is important for the LC to deliver mobile phase compositions reproducibly even under these challenging conditions. As the latest evolution of the ACQUITY UPLC Platform for bioseparations, the ACQUITY Premier System is offered in a Binary Solvent Manager (BSM) configuration to meet these demanding separation requirements while reducing analyte/surface adsorption over previous generations of biocompatible platforms for improved chromatographic performance. Here, the ability of this system to deliver reproducible retention times to support robust peptide mapping workflows is demonstrated.

To evaluate the ACQUITY Premier BSM's performance for real-world applications, the sample analyzed was a NIST humanized monoclonal antibody (mAb) Reference Standard 41116107 tryptic digest. The sample was run across a range of separation conditions that may be encountered during the development and commercialization

of biotherapeutic drug products. An overview of the experimental design is shown in Figure 1. This included conditions at three different flow rates: high flow (0.500 mL/min), moderate flow (0.350 mL/min), and slow flow (0.200 mL/min), and at each flow rate, three gradients, including 1% B/min, 0.5% B/min, and the shallowest 0.25% B/min. At each condition, three replicate injections were used for calculating the standard deviation. These nine method combinations (27 injections) were evaluated on three ACQUITY Premier BSM systems to gauge the intersystem reproducibility.

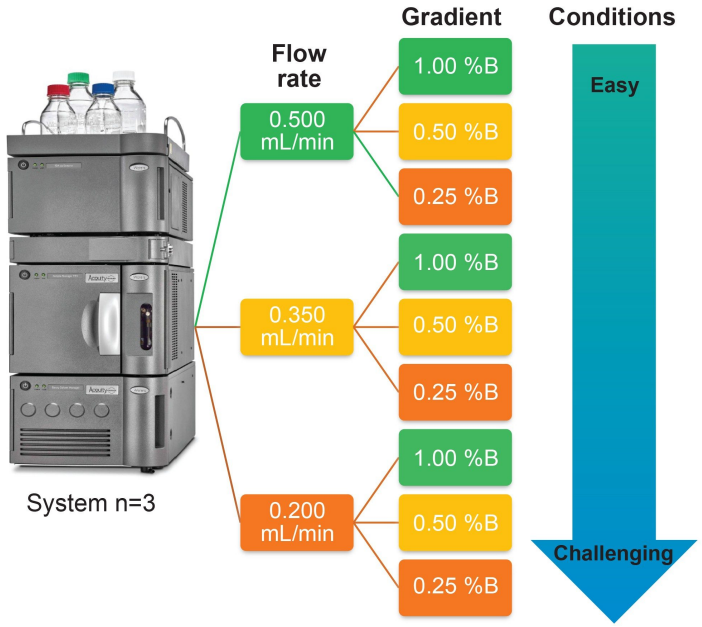

Figure 1. Experimental design for evaluation of intersystem performance across LC conditions.

A total of 18 peaks were selected that were equally sampled across the peptide map to use for retention time reproducibility analysis, as shown in Figure 2. Table 1 shows a list of the *m/z* for each peak that was monitored with SIR-MS and the standard deviation of the retention time across all conditions. The retention time of each peptide was normalized to the mean within each condition to enable comparison across conditions and peaks. The retention time reproducibility shows that intersystem analyses can confidently be performed without sacrificing analytical rigor.

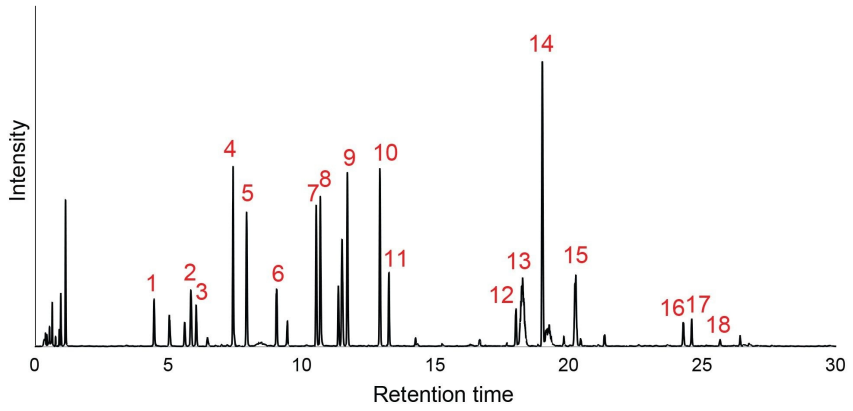

Figure 2. Base peak ion (BPI) chromatogram of NIST mAb digest used in this study with peaks included in analysis indicated by numbers. Peak numbers correlate to information displayed in Table 1.

Table 1. Peak Results: Retention Time Reproducibility.

Peak	Name	*m/z*	Retention time SD	
			min	sec
1	LC:T3	406.4	0.009	0.52
2	HC:T30	419.9	0.008	0.46
3	HC:T21	418.4	0.007	0.41
4	LC:T18	626.4	0.007	0.41
5	LC:T5	476.9	0.009	0.56
6	HC:T11	925.4	0.006	0.36
7	HC:T36	581.7	0.010	0.59
8	HC:T41	561.5	0.010	0.60
9	HC:T23	560.3	0.009	0.51
10	LC:T15	714.2	0.009	0.56
11	HC:T22	752.3	0.010	0.63
12	HC:T37	849.3	0.008	0.49
13	HC:T20	712.4	0.020	1.21
14	HC:T26	603.8	0.011	0.64
15	LC:T11	600.4	0.014	0.83
16	HC:T2	926.6	0.012	0.71
17	HC:T15	960.5	0.015	0.88
18	LC:T7	1122.1	0.012	0.70

SD, standard deviation; LC, light chain; HC, heavy chain; T, trypsin fragment.

Figure 3 shows a grid representing the performance of the system at each specific condition, combining data from all 18 peptides across all three systems. As would be expected, the peptide elution was most reproducible using steep gradients, especially with higher flow rates. As the conditions became more demanding of the binary pump system, retention time standard deviation increased. However, the maximum standard deviation is still approximately 1 second, demonstrating the ability of the system to deliver readily validated peptide mapping assays under these more demanding conditions.

As a comprehensive analysis of performance, retention times from all 18 peptides across all nine conditions and three systems were mean-normalized, plotted as a histogram, and fitted to a Gaussian distribution. Based on the fitted Gaussian distribution, 68% (σ) of the population is within ±0.0057 min, 95% (2σ) is within ±0.0114 min, and

99.7% (3σ) is within ±0.0171 min. These results demonstrate that the ACQUITY Premier BSM is delivering the same high-precision peptide mapping results as previous Waters Binary LC systems.[1–3]

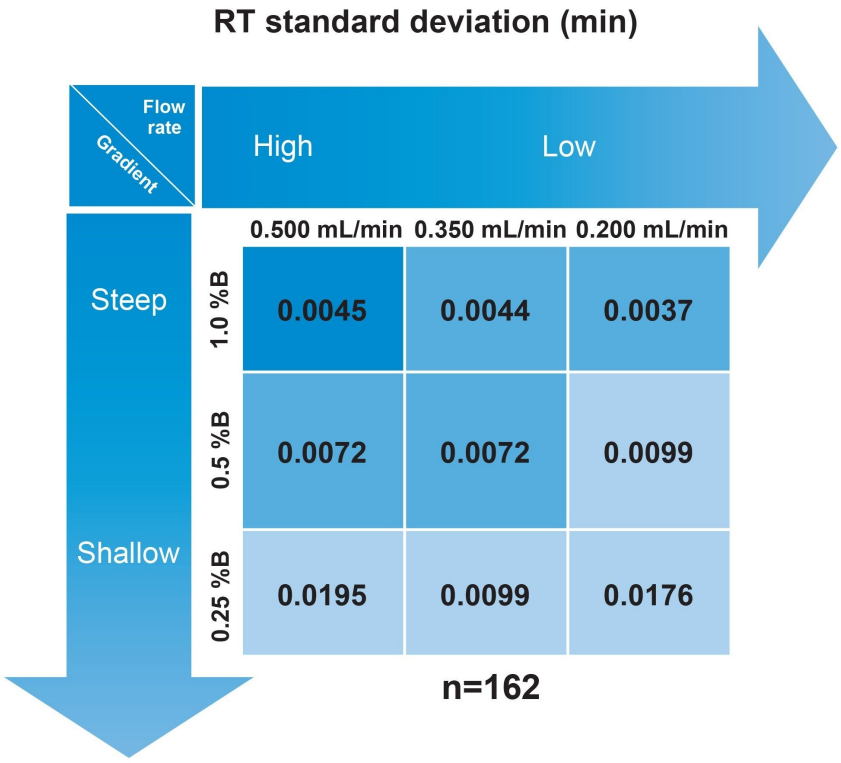

Figure 3. Experimental design and intersystem retention time standard deviation at each of the nine conditions evaluated. Retention times were calculated from 18 peaks measured in triplicate injections on three distinct systems (n = 162).

The remarkable reproducibility of the platform across systems is particularly advantageous, as it streamlines method migration. Methods that are developed on one system can be implemented on another system with minimal intervention. An example of this is shown in Figure 4 with the overlay of three chromatograms of NIST mAb tryptic digest samples run across three ACQUITY Premier BSM UPLC systems. The chromatograms shown represent the most extreme conditions tested, i.e. the fastest flow (0.5 mL/min) with the steepest gradient (1% B/min) and the slowest flow (0.2 mL/min) with the shallowest gradient (0.25% B/min). Under both conditions, the peaks are in good agreement across the three systems without the need for retention time alignment or other

user intervention.

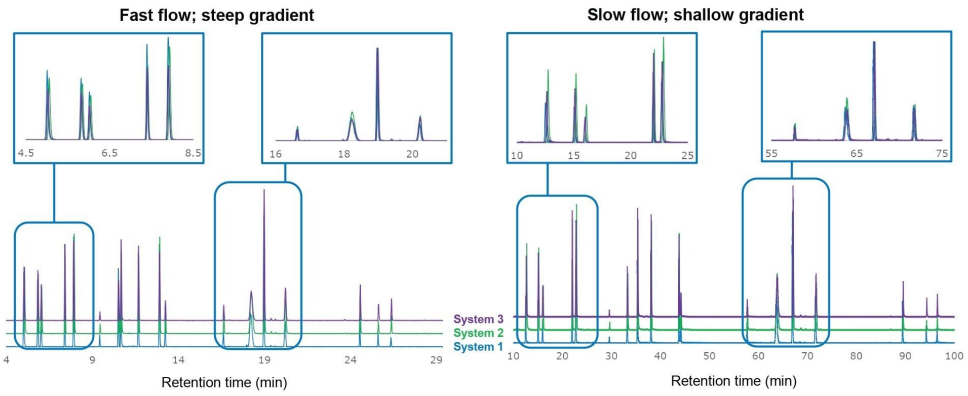

Figure 4. Overlay of injections of NIST mAb digest run on three ACQUITY Premier BSM Systems under two extremes of separation conditions. Left: 0.5 mL/min flow rate and 1% B/min gradient; right: 0.2 mL/min flow rate and 0.25% B/min. Chromatograms are offset by 10% intensity for visualization.

With the retention time inter/intra assay performance of the ACQUITY Premier System established across a broad set of conditions, we sought to assess the performance of the MaxPeak HPS Technology. On high surface area LC components, this novel surface technology is designed to reduce nonspecific peptide adsorption on metal surfaces, improving peak shape, sensitivity, and reproducibility.[4–6] To assess peptide adsorption, seven acidic peptides that are susceptible to adsorption on metal surfaces were selected for analysis. One basic peptide was also included in the assessment to act as a negative control. The peak tailing factor was calculated as defined by USP, where the peak width was divided by double the distance from the front of the peak to the peak apex at 5% peak height (see inset of Figure 5A). Based on this equation, a tailing factor >1 indicates the presence of tailing, and a tailing factor value ≤1.5 is generally deemed as acceptable for RPLC-based peptide mapping assays.

Figure 5A shows the tailing factor of each of the peaks evaluated at three different flow rates (0.5 mL/min, 0.35 mL/min, and 0.2 mL/min). As a comparison, the peaks were also evaluated with a standard stainless steel CSH™ C_{18} column at 0.5 mL/min. The tailing factor was minimal for each peak at all flow rates with the ACQUITY Premier Column and markedly improved from the standard column for most peptides. At the same flow rate, the ACQUITY Premier Column showed up to 83% reduction in peptide peak tailing, as notated in the figure.

Furthermore, the error bars, indicative of standard deviation across 3 replicate injections on each system, are greatly reduced with the ACQUITY Premier Column compared to the standard column, indicating improved reproducibility and quantitative performance.

Another notable advantage of using the MaxPeak HPS Technology is that the reduction of peak tailing reveals low-abundance impurities that would otherwise be obscured by the larger, artificially broadened peak. An example is shown in Figure 5B, where the HC:T37 exhibits substantial tailing with a standard column that obscures the low-abundance peaks eluting after it. With the ACQUITY Premier Column, these low-abundance peaks, identified to be deamidated forms of HC:T37, are both detectable and quantifiable. These results demonstrate the immediate impact high-quality separations can have on peptide mapping assays deployed for targeted attribute and product purity analysis.

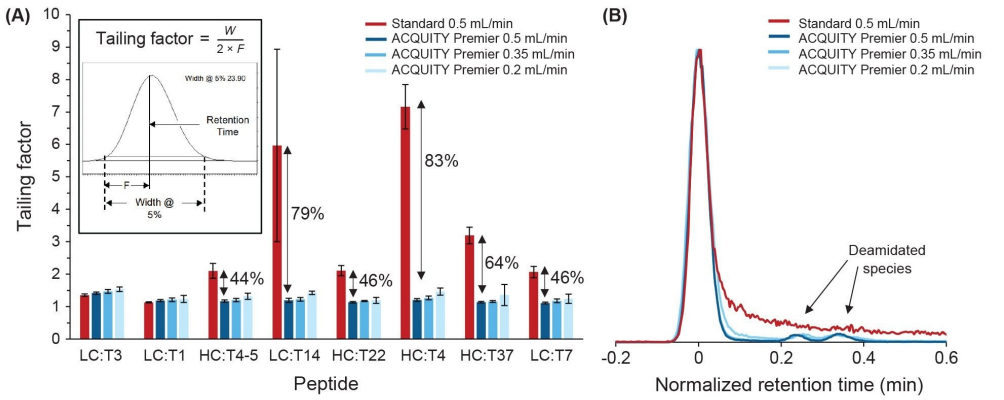

Figure 5. Evaluation of the performance of MaxPeak HPS Technology, including (A) calculated tailing factor for one basic peptide (LC:T3) and seven acidic peptides on a standard CSH C_{18} Column at 0.5 mL/min and an ACQUITY Premier CSH C_{18} Column at three flow rates, and (B) example of how the reduction of tailing with MaxPeak HPS Technology enables detection of low-abundance species, showing HC:T37 and its two low-abundance deamidated forms. Error bars represent standard deviation across three systems with three replicate injections each (n = 9), annotations in (A) indicate percentage reduction in tailing between standard and ACQUITY Premier Columns.

Conclusion

In peptide mapping workflows, reproducible retention times are critical to reducing assay failures and ensuring accurate peak assignment and comparability across analyses. This study showcases the retention time precision of the ACQUITY Premier BSM across systems, even under the most demanding separation conditions. The novel MaxPeak HPS Technology substantially improves chromatographic performance by reducing the effect of peak tailing. The combination of system performance and system bio-inertness will lead to more robust assays that can be more readily transferred and validated, with increased confidence in data interpretation for peptide attribute mapping and product impurity assays.

References

1. Kaffashan A, Wheat TE. ACQUITY UPLC System-to-System Reproducibility for Peptide Mapping. 2009 July, Waters Application Note, 720003175.
2. Simeone J, Hong P, McConville PR. Performance of the ACQUITY UPLC I-Class PLUS System for Methods which Employ Long, Shallow Gradients. 2018 May, Waters Application Note, 720006290.
3. Dao D, Koshel BM, Birdsall, RE, Yu YQ. Peptide Mapping Retention Time Repeatability Under Shallow Gradient Conditions. 2020 December, Waters Application Note, 720007086.
4. DeLano M, Walter TH, Lauber MA, Gilar M, Jung MC, Nguyen JM, Boissel C, Patel AM, Bates-Harrison A, Wyndham KD. Using Hybrid Organic-Inorganic Surface Technology to Mitigate Analyte Interactions with Metal Surfaces in UHPLC. *Anal Chem*. 2021 Apr;93:5773–81.
5. Gilar M, DeLano M, Gritti F. Mitigation of Analyte Loss on Metal Surfaces in Liquid Chromatography. *J Chromatogr A*. 2021 May;462247.
6. Birdsall RE, Kellett J, Ippoliti S, Ranbaduge N, Lauber MA, Yu YQ, Chen W. Reducing Metal-Ion Mediated Adsorption of Acidic Peptides in Rplc-Based Assays Using Hybrid Silica Chromatographic Surfaces. *J Chromatogr B*. 2021 Apr;122700.

Featured Products

ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>

ACQUITY UPLC Tunable UV Detector <https://www.waters.com/514228>

ACQUITY QDa Mass Detector <https://www.waters.com/134761404>

Empower Chromatography Data System <https://www.waters.com/10190669>

720007593, April 2022

© 2024 Waters Corporation. All Rights Reserved.
使用條款 隱私權政策 商標 就業機會 法律和隱私權聲明 Cookie Cookie偏好設定