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Application Note

Peptide Mapping Retention Time Repeatability Under Shallow Gradient Conditions

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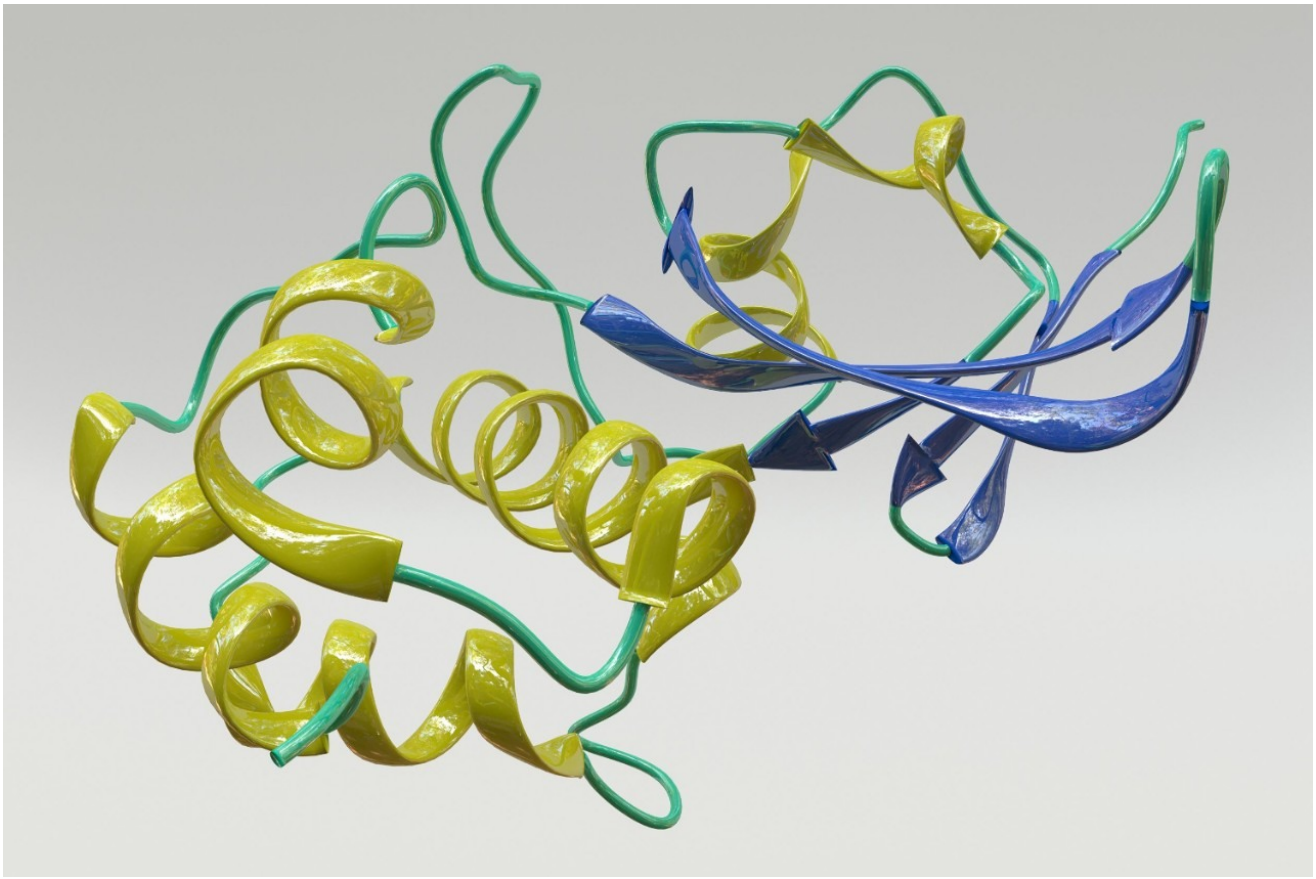

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

Chromatographic methods operating under low flow rates and shallow gradients require LC instrumentation that can deliver precise and accurate gradients to ensure retention time repeatability. Peptide mapping methods often require shallow gradient conditions so that optimal resolution can be achieved for these highly complex samples. In addition, low flow rates support smaller column diameters and are more amenable to electrospray ionization mass spectrometry (ESI-MS), should that be a requirement of the analysis. This work evaluates peptide mapping results collected on a biocompatible binary LC system over 24 hours and compares results over three days. The average standard deviation in retention time of selected peaks is less than 0.5 seconds for each of the three days while the average difference in retention time over the course of the three-day study is less than 3 seconds, demonstrating excellent intra-assay and inter-assay precision.

Benefits

- Superior gradient delivery and performance with the biocompatible ACQUITY UPLC H-Class PLUS Bio Binary System under challenging method conditions
- Exceptional intra- and inter-assay precision demonstrated through highly reproducible retention time results

Introduction

Retention time reproducibility is a critical piece of analytical method development for ensuring that chromatographic peaks can be properly identified and accurately quantitated. Peptide mapping assays are among the most difficult biopharmaceutical methods to establish retention time reproducibility because separation conditions often rely on low flow rates and shallow gradients to achieve the desired resolution. Because of these challenging separation conditions, LC system selection is an important consideration for achieving optimal performance. Solvent delivery, or pump design, can play a key role in peptide mapping repeatability when using low flow rates and shallow gradients. Although quaternary-based LC systems are particularly useful for method development because the additional solvent lines allow separation parameters to be screened more rapidly, binary pumps have a distinct advantage in being able to deliver more precise and accurate gradients. Waters binary pumps are designed to deliver exceptional compositional precision and accuracy under the challenging method conditions often associated with peptide mapping assays. The objective

is this work is to highlight retention time repeatability using a biocompatible binary-based LC system to deliver low flow rates and shallow gradient conditions over the course of a three-day peptide mapping evaluation.

Results and Discussion

To assess the ACQUITY UPLC H-Class PLUS Bio Binary System under challenging gradient conditions, peptide mapping data was compared using consecutive injections over the course of a three-day period. A shallow gradient of 0.41% B/min at 0.200 mL/min was used to separate the Waters tryptic mAb digest standard, a reduced and alkylated tryptic digest of NIST mAb (P/N 186009126 < https://www.waters.com/nextgen/us/en/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>), on a ACQUITY UPLC Peptide CSH C_{18} Column (130 Å, 1.7 µm, 2.1 mm x 100 mm, P/N 186006937 <https://www.waters.com/nextgen/us/en/shop/columns/186006937-acquity-uplc-peptide-csh-c18-column-130a-17--m-21-mm-x-100-mm-1k.html>) using a 135 minute method in 0.1% v/v formic acid in water (mobile phase A) and acetonitrile (mobile phase B). Sample sets consisted of four blank injections followed by three replicate injections of the tryptic digest standard with an equilibration period between sample sets for a total run time of 24 hours per sample set. The digest standard was prepared fresh for daily use.

From overlays of the chromatograms associated with each daily injection series, minimal retention time drift is observed both within a single data set, as well as across the three days (Figure 1). Fourteen peaks were selected throughout the length of the run to further investigate retention time repeatability. Table 1 reports the average retention time and standard deviation for each of the selected peptides across the three-day study. The average standard deviation of retention time is less than 0.5 seconds for each of the three days, which meets instrument specification and shows excellent agreement within each of the data sets. A slight shift to earlier retention time can be seen when comparing Day 3 to Day 1 results. The average difference in retention time between Day 1 and Day 3 is less than 3 seconds, which is considered negligible over the course of the 135-minute method. As this shift in retention time is systematic, the shift is attributed to small changes in mobile phase composition over the course of the study. Furthermore, resolution was not impacted, which is also an important metric for proper peptide identification and quantification. These results demonstrate that the ACQUITY UPLC H-Class PLUS Bio Binary System can provide superior intra- and inter-assay precision throughout an injection series over a multiple day study.

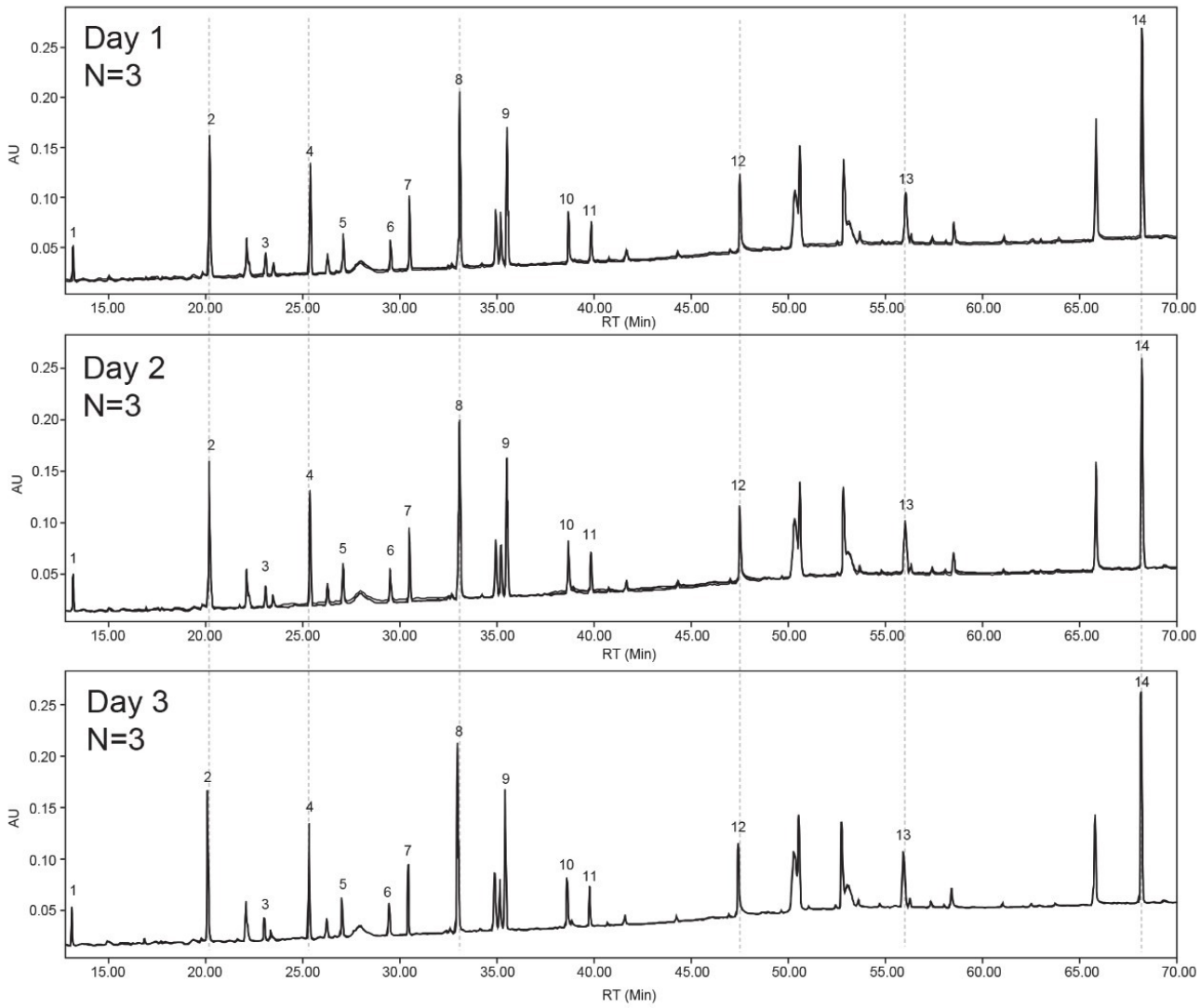

Figure 1. Chromatographic overlays of a triplicate injection series of Waters tryptic digest standard over a three-day evaluation.

	Day 1		Day 2		Day 3	
Peak	RT average	RT std dev.	RT average	RT std dev.	RT average	RT std dev.
1	13.37	0.0096	13.37	0.0049	13.35	0.0067
2	20.37	0.0050	20.36	0.0050	20.33	0.0035
3	23.24	0.0023	23.24	0.0059	23.21	0.0006
4	25.53	0.0026	25.53	0.0081	25.50	0.0051
5	27.23	0.0010	27.22	0.0052	27.19	0.0035
6	29.65	0.0010	29.64	0.0053	29.59	0.0046
7	30.64	0.0038	30.64	0.0055	30.61	0.0061
8	33.19	0.0026	33.18	0.0092	33.14	0.0064
9	35.62	0.0031	35.61	0.0099	35.57	0.0070
10	38.77	0.0038	38.78	0.0108	38.73	0.0072
11	39.94	0.0044	39.94	0.0106	39.89	0.0081
12	47.57	0.0045	47.57	0.0086	47.53	0.0105
13	56.07	0.0032	56.06	0.0056	55.99	0.0182
14	68.19	0.0151	68.19	0.0049	68.11	0.0179

Table 1. Average retention time (min) and standard deviation of selected peptides of a triplicate injection series over a three-day evaluation.

Conclusion

Peptide mapping analyses often employ low flow rates and shallow gradients, conditions that are considered challenging for developing robust analytical methods. Although some variability in retention time is expected under these conditions, retention time repeatability is critical for proper peptide identification and quantitation. This work demonstrates that the ACQUITY UPLC H-Class PLUS Bio Binary System can be used to deliver exceptional gradient precision and to minimize the retention time variability experienced under challenging method conditions.

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720007086, December 2020