Evaluation of Pump Performance for Long Shallow Gradient Peptide Mapping Analysis

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INTRODUCTION

Peptide mapping is a critical tool in biopharmaceutical analysis for characterization and impurity testing. Biotherapeutics are typically complex and contain peptides with a wide range of chemical properties. The samples found in these analyses require demanding method conditions to achieve chromatographic separation, including low flow rates and long, shallow gradients.[1] These are challenging conditions to produce repeatably for high and ultra-high performance liquid chromatography system (HPLC/UHPLC) pumps.[2,3] As a result, accurate and precise pump performance is critical to success with these types of reversed-phase protein digest separation methods. Manufacturers have released systems to address these types of samples, with more robust flow paths better equipped to handle demanding conditions.

In this study, an enolase digestion standard is used as a representative complex sample across multiple HPLC and UHPLC systems designed for bio related applications. Injections were made using a long shallow gradient method where organic content was increased at a rate of 0.5 %/min over 96 minutes. Peaks were selected based on sufficient sensitivity and resolution for quantitative purposes and were limited by resolution differences across systems. The Arc™ Premier System was studied in both binary and quaternary configurations and was compared to other bio LC systems for peak retention time standard deviation and signal-to-noise.

METHODS

Final Method Conditions	
Systems	1) Arc Premier System with binary solvent manager, CH-A column heater and 2998 PDA Detector 2) Arc Premier System with quaternary solvent manager, CH-A column heater and 2998 PDA Detector 3) and 4) Quaternary bio LC System
Detection	214 nm, 10 Hz
Mobile Phase A	0.1% Trifluoroacetic acid in Water
Mobile Phase B	0.1% Trifluoroacetic acid in Acetonitrile
Flow Rate	0.500 mL/min
Sample Temp	10 °C
Injection Volume	25 µL
Column	XSelect™ CSH™ C18 Column 2.5 µm, 4.6 × 150 mm
Column Temp	60 °C

Gradient Table:

Time (min)	%A	%B
Initial	98	2
3.0	98	2
99.0	50	50
104.0	10	90
109.0	10	90
111.0	98	2
131.0	98	2

Sample Preparation:

Vials of Waters MassPREP Enolase Digestion Standard were reconstituted in 100 µL of 0.1% trifluoracetic acid in water (mobile phase A). Vials were pooled for sufficient sample across all systems and redistributed as needed. Mobile phases were also prepared as a batch and distributed among systems to minimize variability. Samples and mobile phases were prepared daily.

RESULTS AND DISCUSSION

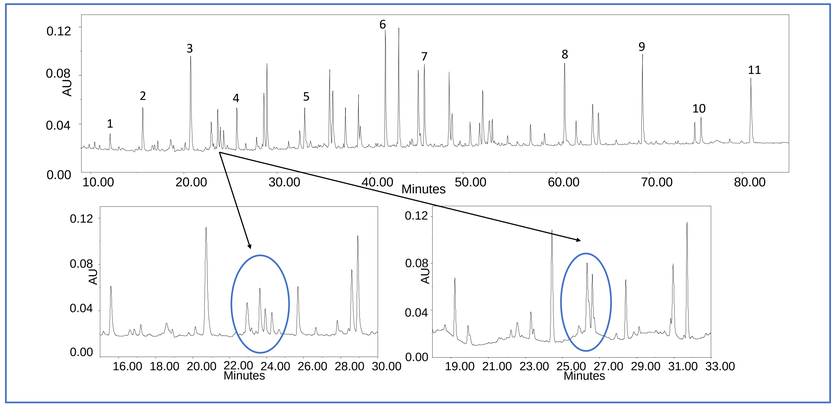

Figure 1. Annotated chromatogram on the Arc Premier Binary System, with numbered peaks used in further analysis. Zoomed area displays 15-minute portion of chromatogram on Arc Premier System (left) and Quaternary bio LC system.

There are several instances of incomplete resolution in the chromatogram appearing in Figure 1. To monitor performance characteristics, a peak should be fully resolved on all systems to ensure accurate comparison. A peak that is not fully resolved will generate less reproducible results both within a sample set and across multiple days of testing. The 15-minute windows below the complete chromatogram show results taken from a sample injection run on the same day on both the Arc Premier Binary System and another quaternary system. The left window shows four distinct peaks in the 22.5 to 25 minute range, while the right shows two peaks in the 26.5 to 29 minute range, with these time differences being explained by factors such as delay volume. For this reason, it is not possible to use any of the four peaks visible in the Arc Premier System chromatogram for any quantitative measurements that would compare to a peak within this range. The peaks showing sufficient resolution across all systems have been labeled in the above chromatogram and are used in further analysis.

Retention time standard deviation and signal-to-noise were measured to monitor pump performance across the eleven peaks of interest. While the system suitability was met for all systems, the Arc Premier System displayed the lowest and most consistent deviation throughout the chromatogram, particularly in the early portion. For peaks 1 through 5, the average error for the quaternary and binary Arc Premier Systems was 0.011 minutes and 0.014 minutes respectively. Quaternary systems 1 and 2 displayed errors of 0.032 and 0.031 minutes. The performance increases for the second half of the peaks on quaternary system 1, while the two Arc Premier Systems and quaternary system 2 remain at similar levels, with average deviations of 0.015 minutes, 0.014 minutes, 0.048 minutes, and 0.016 minutes. This shows the ability of the Arc Premier System to perform an accurate gradient delivery in the most difficult portion of chromatogram, where there is the lowest %B of the gradient.

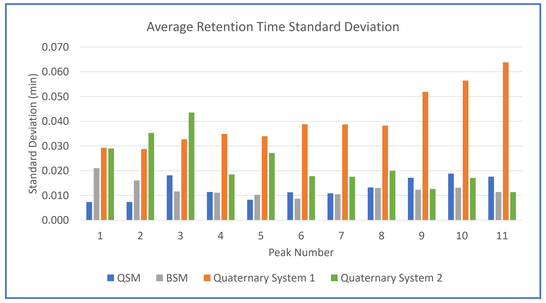

Figure 2. Average retention time standard deviation results obtained on four systems. Averages were first calculated within sample set (six injections) followed by aggregating over four sample sets.

RESULTS AND DISCUSSION

A flat baseline is in integral part to maximizing sensitivity and to achieving consistent integrations. As shown in Figure 1, there is a significant baseline ripple present in one of the quaternary systems that is not present in the Arc Premier System chromatogram. In order to measure the impact of the baseline fluctuation present across the systems, the average signal-to-noise for each peak is measured. The noise range selected was the same across all four systems, with small adjustments due to effects such as gradient delay. As shown below in Figure 3, all systems displayed sufficient signal-to-noise for quantitation across all peaks. The two Arc Premier System configurations displayed the highest signal-to-noise, indicating the lowest noise and thus smallest baseline interference.

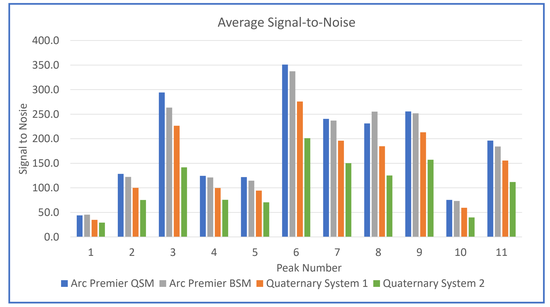

Figure 3. Average signal-to-noise results obtained on four systems. Averages were first calculated within sample set (six injections) followed by aggregating over four sample sets.

CONCLUSION

Peptide mapping analysis requires demanding methods where small changes in system performance can yield a large impact on chromatographic results. All systems showed strong performance, meeting the manufacturer specifications for each parameter studied. The Arc Premier System in both binary and quaternary configurations displays the most consistent baseline and gradient delivery, evidenced by the most reproducible retention time and the highest signal-to-noise among the four systems tested.

REFERENCES

[1]Simeone J, Hong P. Peptide Mapping using Binary Biocompatible LC Systems: Evaluation of Retention Time Precision and Mixing Effects on Waters and Competitive LCs. Waters Application Note, 2021 Mar, 720007078.
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