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Migration of an HILIC Method for Released Glycan Analysis from the ACQUITY UPLC H-Class PLUS Bio System to the Arc Premier System

Robert E. Birdsall, Jacob Kellett, Ying Qing Yu

Waters Corporation

Abstract

Methods are frequently migrated between different LC platforms in the development and manufacturing of biopharmaceuticals. In these instances, comparability studies are typically performed to ensure performance and results are maintained across LC platforms. The Arc Premier System featuring MaxPeak High Performance Surfaces was recently introduced as a flexible LC platform to support development and manufacturing activities. An HILIC method for released glycan analysis showed comparable results when migrated from an ACQUITY UPLC H-Class Bio PLUS System to the Arc Premier System. Differences in peak area percent and relative retention time were minimal between the two platforms. The Arc Premier System showed a three-fold improvement in the recovery of highly sialylated glycan species, demonstrating how MaxPeak High Performance Surfaces can improve confidence in the assay results. The comparable results with the migrated method and the improved recovery of metal sensitive analytes demonstrates that the Arc Premier System is well suited to supporting the analytical needs in the development and manufacturing of biopharmaceuticals.

Benefits

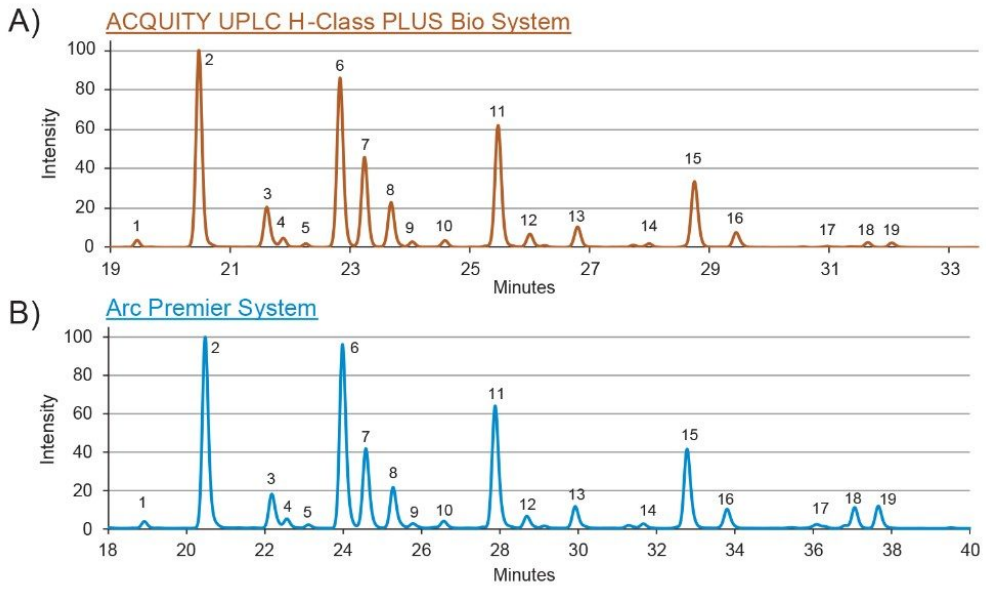
- The Arc Premier System demonstrates its flexibility by achieving comparable performance with a method migrated from an ACQUITY UPLC H-Class PLUS Bio System
- The Arc Premier System with MaxPeak High Performance Surfaces improves the recovery of highly sialylated glycans over traditional stainless-steel systems

Introduction

LC instrument portfolios within the biopharmaceutical industry are generally observed to span a breadth of configurations that vary in performance and specifications. This is in part by design as instrument needs of laboratories will vary across an organization from upstream to downstream activities. When migrating methods between two different LC platforms, differences in performance specifications must be considered as they may impact assay results. Similarly, replacing outdated technology with newer instrumentation can impact assay results due to hardware design and performance differences. In these instances, comparability studies are often performed to ensure results are consistent and performance criteria are maintained across LC platforms. Recently, Waters introduced the Arc Premier System featuring MaxPeak High Performance Surfaces (HPS) Technology. The Arc Premier System is designed as a flexible LC platform that can be readily deployed across labs to support development and manufacturing activities with improved performance towards metal-sensitive analytes. This is accomplished through the introduction of the innovative MaxPeak HPS Technology which is engineered to minimize analyte/surface interaction of metal-sensitive analytes within the LC fluidic path.[1] To demonstrate the Arc Premier System as a suitable LC platform to support drug development and manufacturing activities with improved performance towards metal sensitive analytes, an HILIC method originally developed on the ACQUITY UPLC H-Class PLUS Bio System was migrated to the Arc Premier System. The results of the released glycan analysis were evaluated for performance comparability across the two systems.

Results and Discussion

Migration of methods within or across laboratories is frequently encountered in the development and manufacturing of biopharmaceuticals. As part of the development process, potential critical quality attributes are identified and monitored to ensure the manufacturing process is controlled and reproducible. In this respect, assay results need to be consistent when migrating methods across different LC platforms to ensure results accurately reflect drug product composition. HILIC-based assays are frequently used in both upstream and downstream activities in the characterization and monitoring of glycosylation profiles of therapeutics and thus serves as an ideal case study in the evaluation of the Arc Premier System as a suitable LC platform to support development and manufacturing activity. In this study, an HILIC separation of the Waters *Rapi*Fluor-MS (RFMS) Glycan Standard (p/n: 186007983 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186007983-rapifluor-ms-glycan-performance-test-standard.html>) developed on an ACQUITY UPLC H-Class PLUS Bio System, was used as a representative method to be migrated to the Arc Premier System. To investigate if comparable performance was achievable, the HILIC method was scaled and migrated from the ACQUITY UPLC H-Class PLUS Bio System to the Arc Premier System to accommodate the increased tubing and optimal column format for the Arc Premier System. As shown in Figure 1, comparable glycosylation profiles were achieved between systems using an XBridge Premier BEH Amide Column with a 4.6 mm internal diameter (p/n: 186009946 <https://www.waters.com/nextgen/us/en/shop/columns/186009946-xbridge-premier-glycan-beh-amide-25--m--46-x-150mm-column-1-pk.html>) using an 80-minute scaled method to maintain sample linear velocity. Further evaluation of method equivalency was performed using peak % area and relative retention time for each system.



Gradient

Time	Flow	%A	%B
Initial	0.40	25	75
35.0	0.40	46	54
36.5	0.20	100	0
39.5	0.20	100	0
43.1	0.20	25	75
47.6	0.40	25	75
55.0	0.40	25	75

Gradient

Time	Flow	%A	%B
Initial	1.31	25	75
51.4	1.31	46	54
53.7	0.65	100	0
58.1	0.65	100	0
63.4	0.65	25	75
70.0	1.35	25	75
80.0	1.31	25	75

Figure 1. FLR chromatograms of RFMS Glycan Standard. HILIC method migrated from ACQUITY UPLC H-Class PLUS Bio System to Arc Premier System. The original 35-minute ACQUITY UPLC H-Class gradient was scaled to 51.4 minutes on the Arc Premier System.

As shown in Figure 2, both peak % area and relative retention time are comparable between both systems. The difference in peak % area for all glycan species was within 2%, and low abundant species (peak % area less than 1%) were within 0.1%. The relative retention time values were within +/- two seconds for the scaled method. The Arc Premier System delivered highly consistent results with a percent relative standard deviation less than 1% for peak % area and less than 0.1% for relative retention time. In addition, the Arc Premier System was also observed to have up to a three-fold increase in recovery of the highly sialylated glycans (Figure 1 peaks 17–19). This result was not unexpected, as sialylated glycans exhibit negative charge(s) at a mobile phase pH of 4.5 and are more susceptible to metal-induced adsorption artifacts brought on by analyte/surface interaction.[2] The improved recovery of these metal-sensitive species demonstrate how MaxPeak HPS Technology can be leveraged to improve chromatographic performance and assay results that accurately reflect drug product composition. Collectively, this study demonstrates that the Arc Premier System can deliver comparable performance with a method migrated from the ACQUITY UPLC H-Class PLUS Bio System and is well suited to support the development and manufacturing activity of biopharmaceuticals.

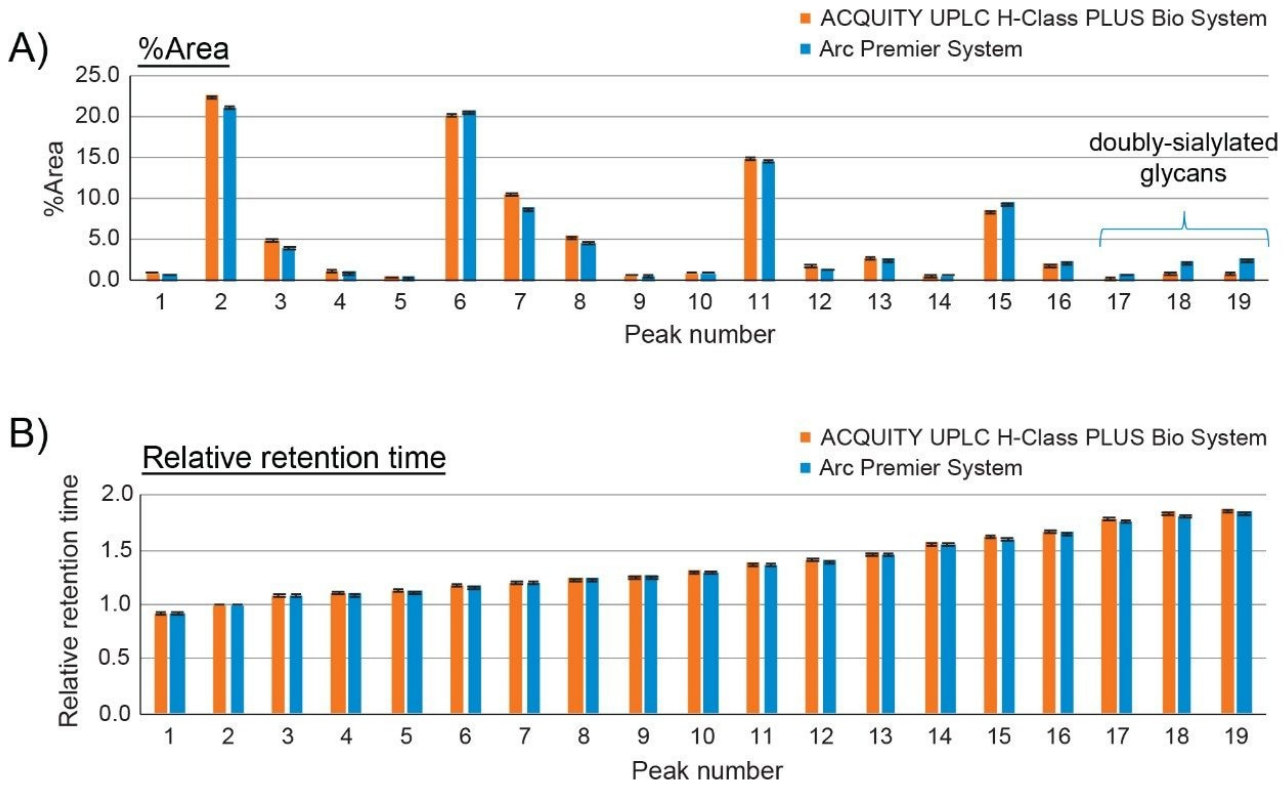


Figure 2. Peak % area and relative retention time comparison between ACQUITY UPLC H-Class PLUS Bio and Arc Premier Systems (N = 5).

Conclusion

Method migration is a key process in the development of biopharmaceuticals and is part of the lifecycle management for analytical procedures. Successful method migration requires that comparable performance is maintained throughout the lifecycle of the method. The Arc Premier System produced consistent peak % area and relative retention time results with an HILIC method migrated from an ACQUITY UPLC H-Class PLUS Bio System. This demonstrates that the Arc Premier System is well suited to support the development and manufacturing activity of biopharmaceuticals.

References

1. M. Lauber, T. H. Walter, M. Gilar, M. DeLano, C. Boissel, K. Smith, R. Birdsall, P. Rainville, J. Belanger, K. Wyndham. Low Adsorption HPLC Columns Based on MaxPeak High Performance Surfaces. Waters Application Note. 720006930EN <https://www.waters.com/webassets/cms/library/docs/720006930en.pdf> , 2021.

2. Ippoliti S, Yu YQ, Chen W. Improved Recovery and Peak Shape of Sialylated O-glycopeptides of Erythropoietin (EPO) with ACQUITY Premier CSH Column. Waters Application Note. 720007227EN, 2021.

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