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Application Note

Demonstrating Method Equivalency of the ACQUITY UPLC H-Class PLUS Bio Binary System and an ACQUITY UPLC I-Class PLUS System Through Method Transfer of a Reversed-Phase Peptide Mapping Method

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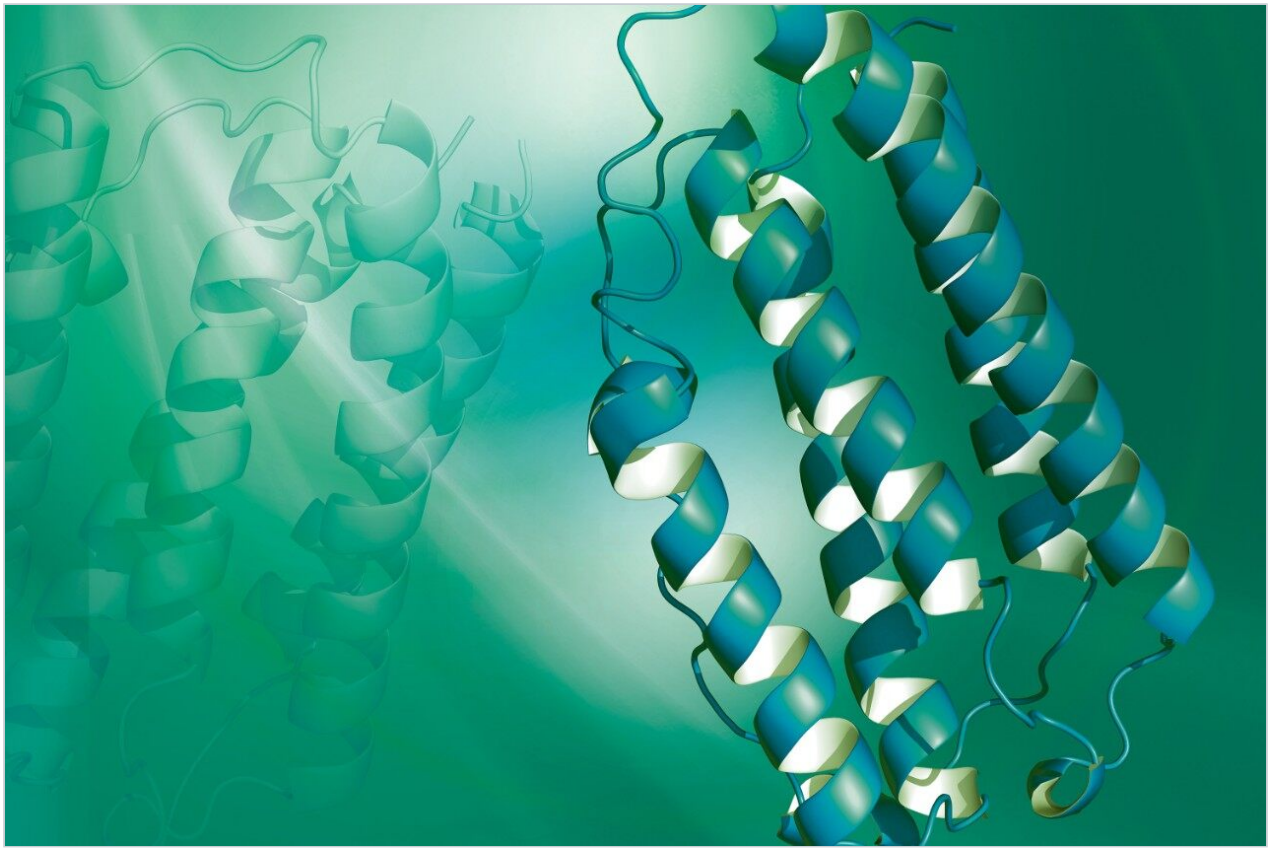

Abstract

With the introduction of more modern LC platforms, it is important to demonstrate that the new system is fit-for-purpose for legacy methods and that method equivalency can be established between the new system and original platform. In this work, a RPLC peptide mapping method was used to evaluate a method transferred from an ACQUITY UPLC I-Class PLUS System to an ACQUITY UPLC H-Class PLUS Bio Binary System where retention time and peak area reproducibility were assessed between the two systems. Chromatographic performance was observed to be similar between systems when systems were run using their standard shipping configurations. The average retention time difference between systems for 13 peptide peaks was approximately 1.5 seconds. However, because LC systems often differ in dwell volume due to differences in fluidic tubing diameter or configuration (e.g. solvent mixer volume), retention time shifts are expected to occur. To account for these differences in an example where system dwell volume is different between LC platforms, Gradient SmartStart Technology can be used to adjust the gradient prior to injection to align retention times without making changes to the gradient table. Chromatographic performance was again observed to be similar for both systems when a gradient offset was applied. An orthogonal plot of the

retention time of the 13 peaks showed an R^2 value of 0.99996, which indicates selectivity was conserved between platforms. Peak area percent was also maintained between systems. These results demonstrate the ability to transfer existing methods between two LC systems to achieve similar chromatographic and quantitative results.

Benefits

- Impact of variable mixer volumes on retention time can be adjusted with Gradient SmartStart Technology in Empower to align retention time between LC systems
- Method equivalency demonstrated through retention time and peak area reproducibility between two binary UPLC systems

Introduction

The analysis of biotherapeutics continues to grow more challenging due to increasing sample complexity and the continuous evolution of new modalities. Developing robust analytical methods is critical for ensuring that any data generated is reproducible, which is especially true as analytical methods are transferred to external contract organizations or internally to QC and manufacturing facilities. Analytical methods are often transferred between multiple vendor platforms but should also accommodate the introduction of new technologies as older instrument platforms are phased out. Accounting for performance differences between LC platforms is a critical piece of a more holistic effort to understand differences between the sending and receiving laboratories.

In this work, a RPLC method for peptides is transferred across two different binary UPLC platforms - a stainless steel ACQUITY UPLC I-Class PLUS System and an ACQUITY UPLC H-Class PLUS Bio Binary System – to evaluate method equivalency under the conditions tested. “Biocompatible” or “bioinert” LC systems are of particular interest to the biopharmaceutical industry because they are designed with materials that resist corrosion and/or contain metal-free flow paths to mitigate surface interaction with proteins and other biological samples. While corrosion-resistant materials may be more geared towards applications such as SEC or IEX, many laboratories do not have dedicated instruments and these same LC systems are used for RPLC separations. The ACQUITY UPLC H-Class PLUS Bio Binary System, a biocompatible binary LC system, takes advantage of a biocompatible flow-path and larger tubing inner diameter, which are more suitable for biological matrices and mobile phase conditions, as well as a binary pump for more reliable

gradient delivery.

Experimental

Sample Description

Waters tryptic digest standard (p/n: 186009126 <https://www.waters.com/nextgen/us/en/shop/standards--reagents/186009126-mab-tryptic-digestion-standard.html>), a reduced and alkylated tryptic digest of NIST mAb reference material, was reconstituted using 0.1% v/v formic acid in water and vortexed for 60 seconds to achieve a final concentration of 0.5 µg/µL. All samples were prepared just prior to injection.

LC Conditions

LC systems:	ACQUITY UPLC H-Class PLUS Bio Binary System (Biocompatible) ACQUITY UPLC I-Class PLUS System (Stainless Steel)
Detection:	214 nm
Vials:	QuanRecovery with MaxPeak HPS Vials
Column(s):	ACQUITY UPLC BEH C_{18} Column, 130 Å, 1.7 µm, 2.1 x 100 mm
Column temp.:	60 °C
Sample temp.:	10 °C
Injection volume:	10 µL
Flow rate:	0.5 mL/min
Mobile phase A:	0.1% v/v FA in Water

Mobile phase B: 0.1% v/v FA in Acetonitrile

Gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
Initial	0.500	99.0	1.0	6
1.30	0.500	99.0	1.0	6
24.70	0.500	60.0	40.0	6
27.00	0.500	35.0	65.0	6
30.30	0.500	35.0	65.0	6
33.00	0.500	99.0	1.0	6
40.00	0.500	99.0	1.0	6

Data Management

Chromatography software: Empower 3 FR4

Results and Discussion

To assess method transfer, a generic RPLC method for peptide mapping was run on a stainless steel binary UPLC system dedicated to RPLC applications, the ACQUITY UPLC I-Class PLUS System, and an ACQUITY UPLC H-Class PLUS Bio Binary System. Both binary LC systems were evaluated using their default shipping configurations. The LC systems produced similar chromatography for 13 peaks selected throughout the length of the gradient (Figure 1). The retention time of the peaks are closely aligned without any manual offset applied, indicating that both systems have very similar gradient delay volumes. Table 1 reports the average retention time of the selected peaks over four injections. From Table 1, the average difference in

retention time is calculated as 1.5 seconds. This corresponds to a difference in dwell volume of 12.5 µL, which is negligible and aligns with the difference in dwell volume of each system as determined through an independent experiment.[1] When evaluating intra-system data, the average standard deviation of retention time of the 13 peaks was 0.0017 (ACQUITY UPLC I-Class PLUS System) and 0.0012 (ACQUITY UPLC H-Class PLUS Bio Binary System). Both systems yielded retention time data that met instrument specification while also producing data comparable to one another to meet application requirements.

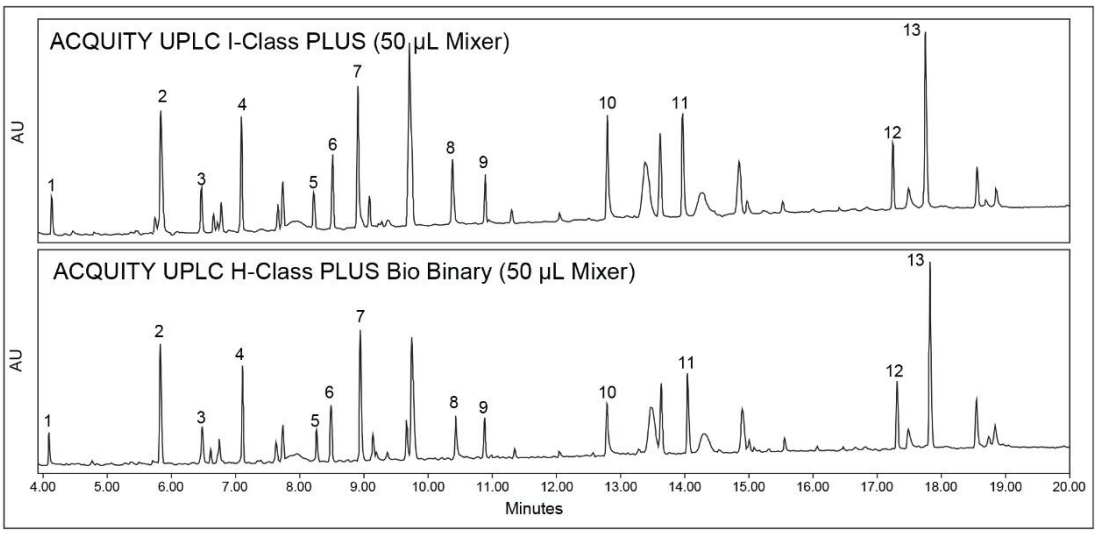

Figure 1. RPLC chromatograms of the NIST mAb tryptic digest standard using default shipping configurations. The dwell volume of each system was determined to be 89.4 µL (ACQUITY UPLC I-Class PLUS System) and 95.8 µL (ACQUITY UPLC H-Class PLUS Bio Binary System) as determined through the method outlined by Hong and McConville.[1] These values meet instrument specification and support the similar retention times generated in the chromatograms. The chromatographic profile is largely preserved between systems, but some variability can be attributed to differences in system-preparedness protocol.

	ACQUITY UPLC I-Class PLUS (N=4)				ACQUITY UPLC H-Class PLUS Bio Binary (N=4)		
Peak no.	RT Avg	RT SD	RT %RSD		RT Avg	RT SD	RT %RSD
1	4.10	0.0019	0.046		4.06	0.0013	0.032
2	5.81	0.0024	0.041		5.80	0.0017	0.029
3	6.44	0.0017	0.027		6.46	0.0013	0.019
4	7.06	0.0024	0.033		7.08	0.0013	0.018
5	8.20	0.0014	0.017		8.24	0.0013	0.016
6	8.49	0.0026	0.031		8.47	0.0010	0.011
7	8.88	0.0017	0.020		8.92	0.0014	0.016
8	10.36	0.0013	0.012		10.41	0.0013	0.012
9	10.87	0.0022	0.020		10.86	0.0008	0.008
10	12.78	0.0021	0.016		12.77	0.0015	0.012
11	13.95	0.0010	0.007		14.03	0.0006	0.004
12	17.23	0.0005	0.003		17.30	0.0006	0.003
13	17.74	0.0013	0.007		17.82	0.0015	0.008

Table 1. Comparison of average retention time (min) of 13 peptides (Figure 1) across four injections.

It is not uncommon to observe shifts in retention time due to dwell volume differences between LC systems when running the same method. Our previous work has shown the benefit of a larger mixer volume for peptide mapping applications.[2] To observe a scenario where a larger mixer might be used and dwell volume is significantly different between LC platforms, the standard 50 µL mixer on the ACQUITY UPLC H-Class PLUS Bio Binary System was replaced with a 340 µL mixer and the same peptide mapping method was run as previously outlined. Although the chromatographic profiles of the ACQUITY UPLC I-Class PLUS System (50 uL mixer) and the ACQUITY UPLC H-Class PLUS Bio Binary System with the 340 uL mixer are similar, a much larger shift in retention time is now observed (Figure 2A). Often the acceptance criteria of the analytical method will report relative retention time to account for LC systems having different dwell volume, but Gradient SmartStart Technology can also be applied to adjust retention time without making changes to the gradient table. In this example, the difference in dwell volume between the two systems was approximately 296 µL. With Gradient SmartStart Technology, this volume can now be used to delay the start of the gradient prior to injection (Figure 2B). This adjustment now aligns the retention time of peaks between the standard ACQUITY UPLC I-Class PLUS System configuration and the ACQUITY UPLC H-Class PLUS Bio Binary System with the 340 µL inline (Figure 2A).

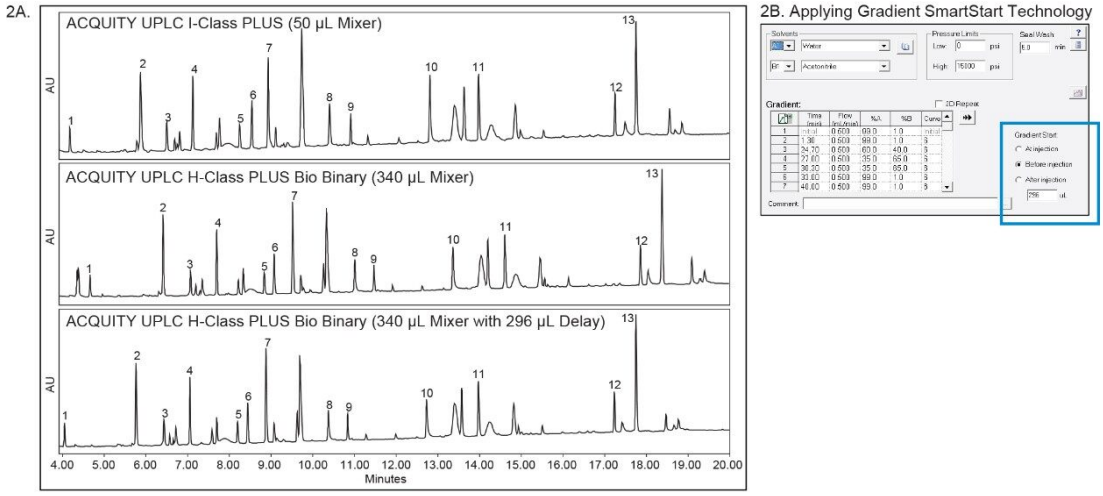

Figure 2A. Chromatograms depicting method transfer using Gradient SmartStart Technology in the Empower Software to account for systems having different dwell volumes. Figure 2B. The gradient start can be applied at injection, before injection, or after injection and is entered as a volume in µL in the Empower Software. This value can be determined through calculating the difference in system dwell volume as determined through the method outlined by Hong and McConville[1] or within the experiment by RT offset (min) x flow rate (mL/min) where the RT offset is determined by comparing the top and middle chromatograms in Figure 2A. Both values align with the more general calculation to determine the difference in mixer volume 340 µL–50 µL = 290 µL. The bottom chromatogram is the result of the gradient start applied before the injection.

To further interrogate this data, Figure 3 plots the retention time of the 13 selected peaks over four replicate injections on both systems against one another to observe any changes in selectivity. The R^2 value is 0.999996 indicating selectivity was highly comparable between platforms for this application. Peak area reproducibility is another metric that is expected to be preserved with a successful method transfer. The peak area percentage of the 13 peptides is reported in Figure 4. Peak area values were similar across both systems. The difference in peak area was less than 1 percent for all peptides (Peak 7 ~1.2%), and for several peptides the difference was 0.1% or less. Although smaller standard deviation and %RSD were observed on the ACQUITY UPLC H-Class PLUS Bio Binary System for most peptides, both systems met instrument specification and demonstrated comparable results to one another.

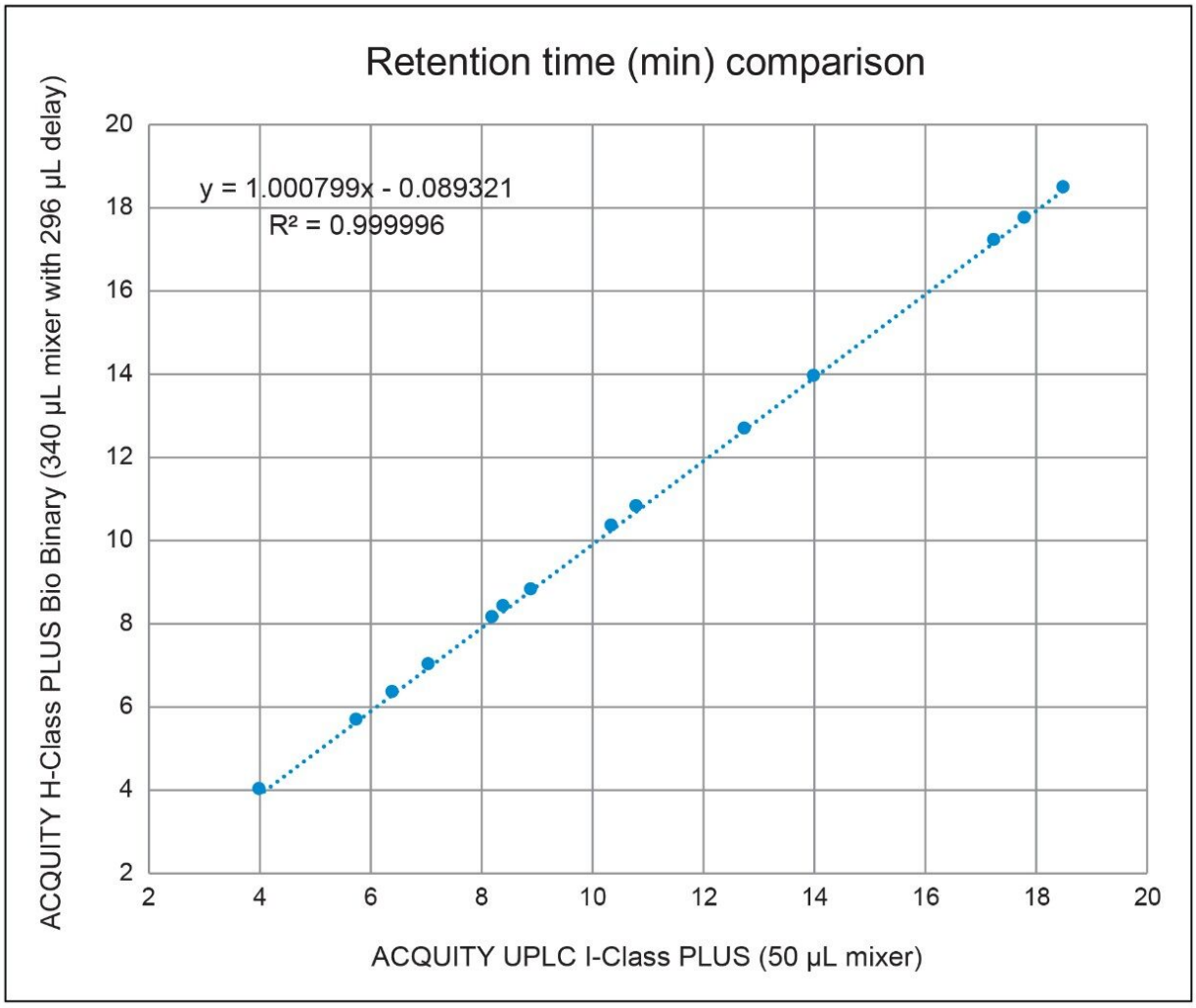

Figure 3. Raw retention time (min) comparison of the ACQUITY UPLC H-Class PLUS Bio Binary System (with 340 µL mixer and gradient delay) and ACQUITY UPLC I-Class PLUS System. Data indicates that selectivity is preserved between the systems.

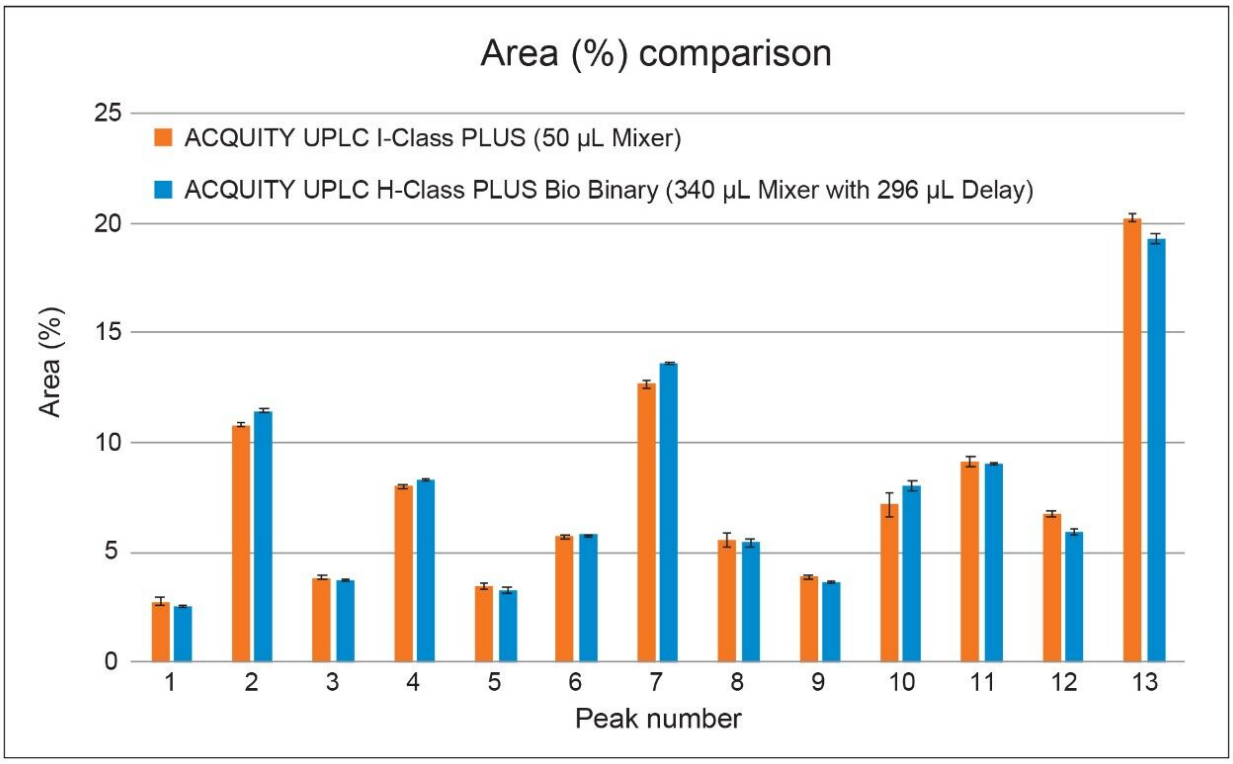

Figure 4. Area (%) comparison of the ACQUITY UPLC I-Class PLUS System and ACQUITY UPLC H-Class PLUS Bio Binary System (with 340 µL mixer and gradient delay) over four injections. Data indicates that peak area is preserved between the systems.

Conclusion

Biopharmaceutical companies rely on robust LC systems to deliver reliable results in the production and testing of pharmaceutical products. The ACQUITY UPLC H-Class PLUS Bio Binary System takes advantage of a biocompatible flow path and a binary pump for more precise and accurate gradient delivery. Method transfer from an ACQUITY UPLC I-Class PLUS System platform to the ACQUITY UPLC H-Class PLUS Bio Binary System demonstrated that equivalent retention time and peak area results could be achieved. Gradient SmartStart Technology was also shown to align retention time between systems in an example where the dwell volume was different between the LC platforms. The tight standard deviation and %RSD values reported demonstrate excellent intra-assay precision but also show that results between systems were highly similar.

References

1. Hong, P, McConville, PR. Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer. Waters White Paper 720005723EN < https://www.waters.com/webassets/cms/library/docs/720005723en.pdf> . 2016 May.

2. Dao, D, Birdsall, RE, Yu, YQ. Instrument Mixer Considerations to Improve Assay Reproducibility and Sensitivity. Waters Application Note 720007011EN < https://www.waters.com/webassets/cms/library/docs/720007011en.pdf> . 2020 Sep.

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