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Method Scaling from ACQUITY™ Premier to Arc™ Premier System

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Abstract

Instruments capable of delivering consistent and reproducible results are highly desirable for efficient development and manufacturing of drug products. Waters™ MaxPeak™ Premier LC Systems in conjunction with Waters Columns Calculator help streamline this process through robust LC performance and automation of method scaling considerations when migrating methods across labs or instruments. In this application note, a peptide mapping method for infliximab was scaled using the Waters Columns Calculator from an ACQUITY™ Premier System (UPLC) to an Arc™ Premier System (UHPLC). Results demonstrate, when scaled appropriately, separation selectivity and mass balance can be conserved across methods for efficient migration of methods. In addition, the MaxPeak™ High Performance Surfaces (HPS) Technology was shown to substantially improve chromatographic performance in terms of peak tailing by reducing nonspecific peptide adsorption, enabling accurate detection and quantitation of lower-abundance impurities for increased assay robustness. Collectively, these results demonstrate the Waters MaxPeak Premier Systems with MaxPeak™ High Performance Surfaces, are well suited for deployment in the development and manufacturing of biopharmaceuticals.

Benefits

- Waters Columns Calculator enables efficient method migrating by automating calculations when scaling

methods across systems or columns

- The ACQUITY™ and Arc™ Premier Systems deliver consistent results enabling robust analyses
- MaxPeak™ High Performance Surfaces reduce undesirable analyte/surface interactions for improved chromatographic performance

Introduction

Analytical needs of supporting labs in the development and manufacturing of drug products depend in part on the criteria of the assay or methods used in the analysis of biopharmaceuticals. In this respect, instruments that can be broadly deployed or can easily support transfer of methods are highly desirable.[1] The Arc™ Premier System is designed as a flexible LC platform that can support development and manufacturing activities. As part of the MaxPeak Premier System portfolio, the Arc™ Premier System is engineered with Waters innovative MaxPeak™ High Performance Surfaces (HPS) Technology to minimize analyte/surface interaction of metal-sensitive analytes for increased chromatographic performance.[2]

In this comparative study, a peptide mapping method will be used as a representative method frequently deployed in both environments to demonstrate the value the Arc™ Premier System brings to the lab and its ability to support development and manufacturing activity. To help automate the process, the Waters Columns Calculator will be used to scale the method from an ACQUITY™ Premier System to an Arc™ Premier System. Percent peak area, relative retention time, and peak tailing of critical peptides will be used as metrics to demonstrate method equivalency.

Experimental

Sample Description

A monoclonal antibody (mAb)-based therapeutic, infliximab, was used for this study to generate a peptide map profile for evaluation purposes. The mAb was reduced, alkylated, desalted, tryptic digested, and acidified. The final sample was reconstituted in 300 µL mobile phase A (0.4 µg/µL). Mobile phase A was used for blank

injections.

ACQUITY™ Premier LC/UV Conditions

LC system:	ACQUITY™ Premier UPLC System (BSM)
Detection:	ACQUITY Premier TUV; 10 mm analytical flow cell; λ = 214 nm
Vials:	QuanRecovery™ Vials with MaxPeak™ High Performance Surfaces, (p/n: 186009186)
Column(s):	ACQUITY™ Premier Peptide CSH™ C_{18} 1.7 µm, 2.1 × 100 mm, (p/n: 186009488)
Column temperature:	60.0 °C
Sample temperature:	10.0 °C
Injection volume:	5.0 µL
Flow Rate:	0.200 mL/min
Mobile phase A:	Water, 0.1% Formic Acid v/v
Mobile phase B:	Acetonitrile, 0.1% Formic Acid v/v
Gradient:	*See below*

Arc Premier LC/UV Conditions

LC system:	Arc™ Premier UHPLC System (BSM)

Detection:	Arc Premier TUV; 10 mm analytical flow cell; λ= 214 nm
Vials:	QuanRecovery™ Vials with MaxPeak™ High Performance Surfaces, (p/n: 186009186)
Column(s):	XSelect™ Premier Peptide CSH C_{18} 2.5 µm, 4.6 × 100 mm, (p/n: 186009908)
Column temperature:	60.0 °C
Sample tempertaure:	10.0 °C
Injection volume:	24 µL
Flow rate:	0.653 mL/min & 0.960 mL/min
Mobile phase A:	Water, 0.1% Formic Acid
Mobile phase B:	Acetonitrile, 0.1% Formic Acid
Gradient:	*See below*

Gradient Table ACQUITY Premier: original method

Time (min)	Flow (mL/min)	%A	%B	Curve
initial	0.200	99.0	1.0	initial
1.00	0.200	99.0	1.0	6
51.00	0.200	65.0	35.0	6
57.00	0.200	15.0	85.0	6
61.00	0.200	15.0	85.0	6
67.00	0.200	99.0	1.0	6
80.00	0.200	99.0	1.0	6

Gradient Table Arc Premier: scaled by gradient

Time (min)	Flow (mL/min)	%A	%B	Curve
initial	0.653	99.0	1.0	initial
1.47	0.653	99.0	1.0	6
75.00	0.653	65.0	35.0	6
83.82	0.653	15.0	85.0	6
89.71	0.653	15.0	85.0	6
98.53	0.653	99.0	1.0	6
117.65	0.653	99.0	1.0	6

Gradient Table Arc Premier: custom by flow rate

Time (min)	Flow (mL/min)	%A	%B	Curve
initial	0.960	99.0	1.0	initial
1.00	0.960	99.0	1.0	6
50.98	0.960	65.0	35.0	6
56.98	0.960	15.0	85.0	6
60.98	0.960	15.0	85.0	6
66.97	0.960	99.0	1.0	6
79.97	0.960	99.0	1.0	6

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

Data Management

Chromatography software:	Empower™ 3, FR5

Results and Discussion

The Waters Columns Calculator is available to users to help automate the calculations performed during the scaling process when methods are deployed on different systems or column formats to allow users to obtain equivalent results. As shown in Figure 1A, the Waters Columns Calculator only requires the original method gradient, column dimensions being used on both systems, and the dwell volume of each system. Using the method, previously described, the dwell volume for each system was determined to be 0.188 mL and 0.283 mL for the ACQUITY™ and Arc™ Premier Systems, respectively (Figure 1B).[3] Using this information, the Waters Columns Calculator can calculate and recommend method changes to preserve chromatography based on scaling factors selected by the user. In this example, an 80-minute peptide mapping method performed on an ACQUITY™ Premier System using an ACQUITY™ Premier Peptide CSH C_{18} Column (1.7 µm, 2.1×100 mm) was scaled to the Arc™ Premier System with the separation performed on an XSelect™ Premier Peptide C_{18} Column (2.5 µm, 4.6×100 mm). As shown in Figure 1A, when selecting the "scale" option, the Waters Columns Calculator will adjust the flow and gradient time to ensure the number of column volumes of solvent is maintained between methods, thus preserving the selectivity of the separation. An example of this is shown in Figure 2 for the peptide mapping method. In this example, Figure 2A represents the original peptide mapping method and Figure 2B represents the migrated method using the "scale" option. As shown in the figure, while the run time of the method was extended to ~120 minutes, the selectivity of the separation was preserved between the migrated method and the original method. As shown in Table 1, further investigation using a selection of peaks as annotated in the chromatogram indicates both systems were able to reliably reproduce the peptide profiles with retention time SD below 0.01 while maintaining separation selectivity between methods with relative retention times (RRT) below 0.02. Alternatively, users are offered the ability to use a "custom" flow if desired to scale the method for the dimension of the column disregarding the particle size. This can be beneficial when higher throughput is needed and users want to maintain the original methods run time while preserving the selectivity of the separation. An example is shown in Figure 2C. As shown in this example, the run time of the method was maintained at 80-minutes with the flow set at 0.960 mL/min to maintain the same column volume of solvent delivered across the gradient. Using this approach, the "custom" method was able to reproducibly perform the separation with retention time SD below 0.02 and absolute retention times matching closely to the original method while maintaining separation selectivity as before with RRTs below 0.02.

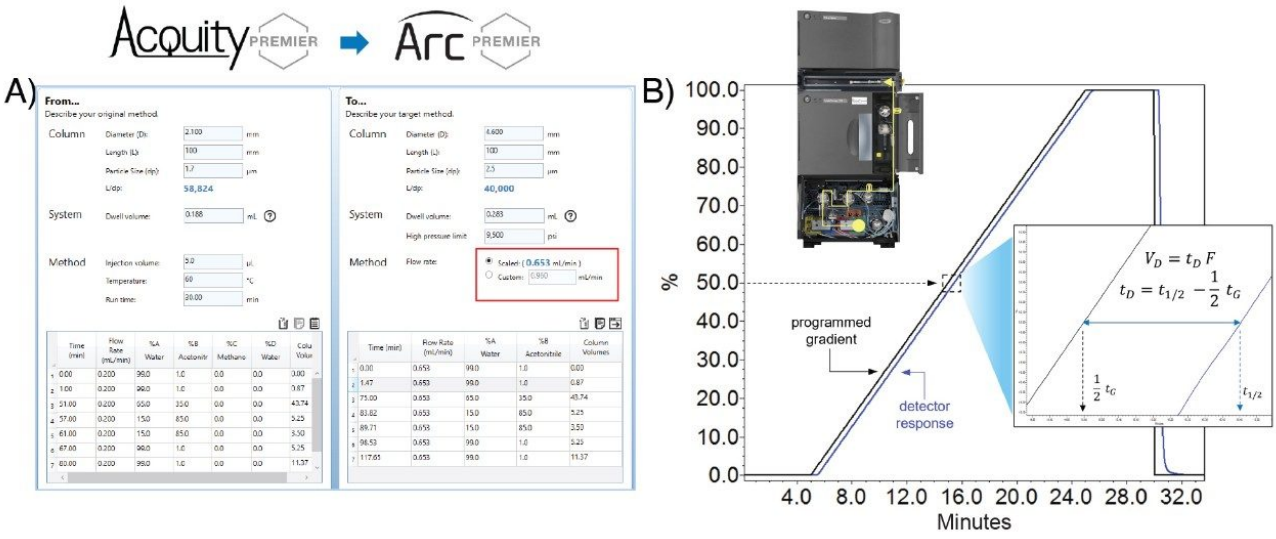

Figure 1: A) Scaling a method using the Waters Columns Calculator. B) Dwell volume, as indicated by the yellow trace on system schematic, measured as a function of detector response and programmed gradient.

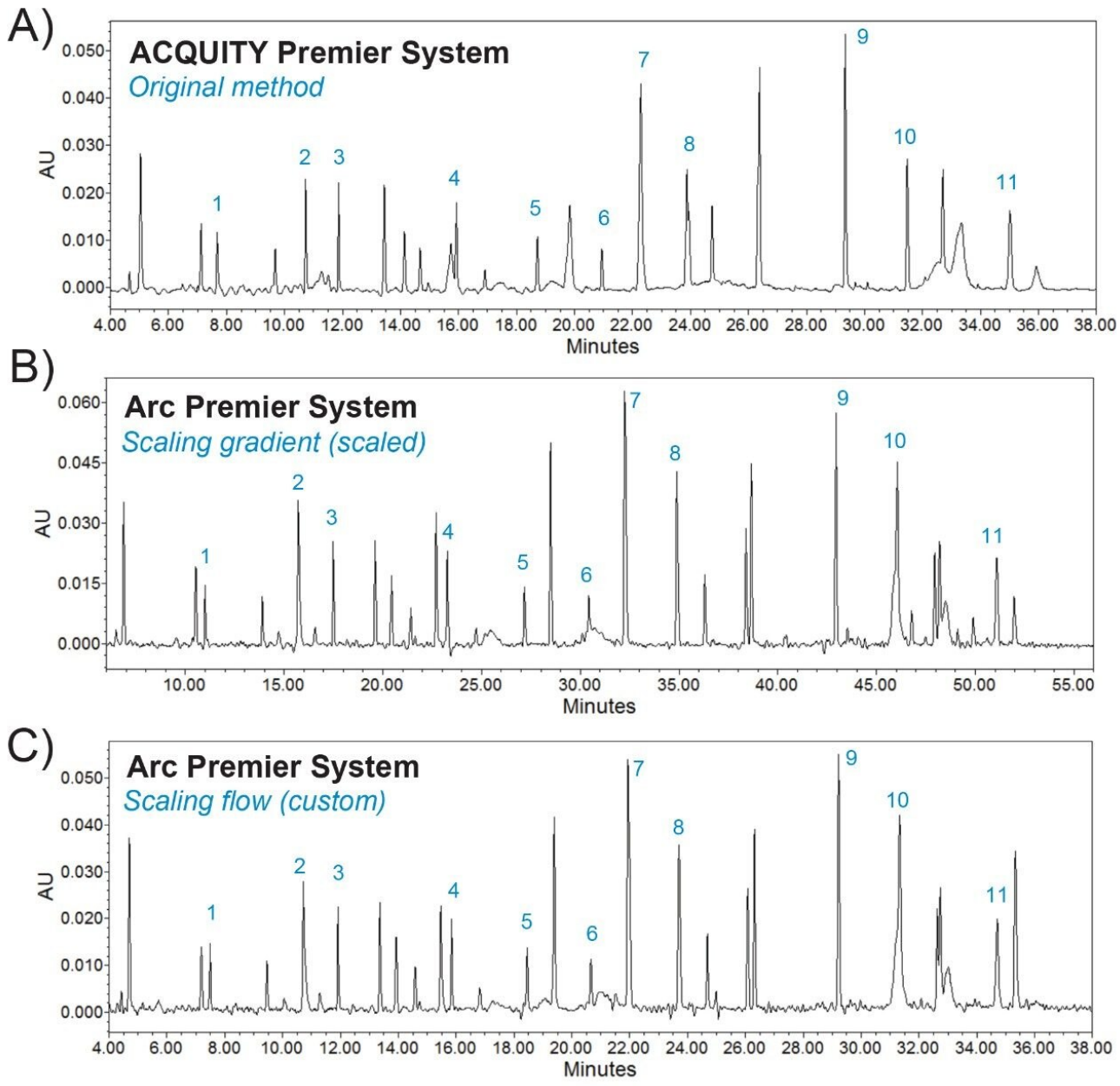

Figure 2: UV absorption spectra for the A) original method on ACQUITY Premier System scaled to the Arc Premier System using the B) "scaled" option and C) and "custom" option with the Waters Columns Calculator. Baseline subtraction was applied to the Arc Premier System data to facilitate easier comparison of chromatograms.

Peak (N=4)	ACQUITY Premier System (original method)		Arc Premier System (custom)		Arc Premier System (scale)		Arc Premier System RRT Δ (peak 6)	
	Mean RT (min)	SD	Mean RT (min)	SD	Mean RT (min)	SD	custom	scale
1	7.680	0.003	7.490	0.001	10.993	0.002	-0.004	-0.004
2	11.865	0.002	11.919	0.001	17.492	0.004	0.011	0.010
3	13.447	0.003	13.366	0.001	19.610	0.003	0.006	0.005
4	15.928	0.004	15.842	0.001	23.259	0.005	0.007	0.006
5	18.726	0.006	18.454	0.002	27.164	0.005	0.001	0.002
6	22.287	0.007	21.944	0.002	32.249	0.005	0.000	0.000
7	23.956	0.009	23.699	0.001	34.879	0.007	0.005	0.007
8	24.749	0.006	24.682	0.001	36.303	0.009	0.015	0.015
9	29.342	0.007	29.220	0.002	42.937	0.012	0.015	0.015
10	31.478	0.008	31.329	0.003	46.035	0.014	0.016	0.015
11	35.020	0.011	34.700	0.001	51.078	0.009	0.010	0.013

Table 1: Evaluation of retention time with migrated methods.

An added benefit of the Arc Premier System is its ability to support LC/MS separations as part of current or future development/manufacturing needs. In this instance, the ACQUITY QDa was used as a form-factor mass detector designed with a small footprint that allows it to be configured in-line to acquire mass data with MS-compatible techniques and is compatible with both the ACQUITY Premier and Arc Premier Systems. An example is shown in Figure 3A for mass data acquired with the ACQUITY QDa using the Arc Premier System running the higher flow rate (0.960 mL/min) method. In this instance, the QDa was connected in-line with the TUV. However Waters offers users the ability to configure the ACQUITY QDa with a divert valve or flow splitter to accommodate higher flow rates or reduce sample matrix effects for increased performance. The Empower CDS integrated MS tools allow users to probe/process complementary mass data. An example is shown in Figure 3B where extracted ion chromatograms (XICs) of the previously discussed peaks were used to evaluate the ACQUITY QDa's ability to deliver consistent mass data under different gradients and flow rates. As shown in Table 2, the ACQUITY QDa was able to deliver consistent results in terms of detector response (peak area) under both the "scaled" and "custom" methods when compared to the original method. Both methods were able to achieve comparable performance in terms of peak area % RSD, which were predominantly below 5% and well within system specification.

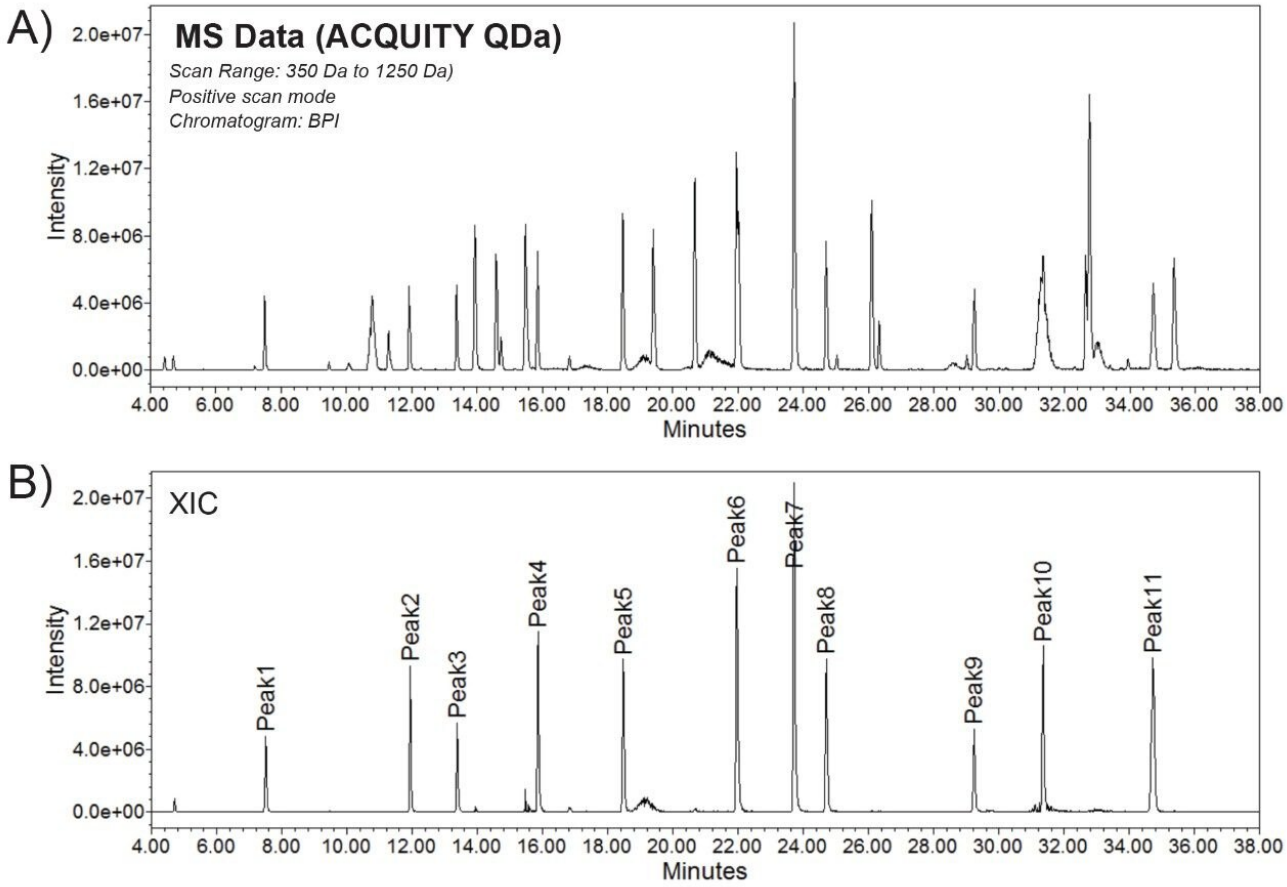

Figure 3: A) BPI and B) XIC of mass data acquired for a peptide map of infliximab using the ACQUITY QDa mass detector configured in-line with an Arc Premier System.

Peak (N=4)	ACQUITY Premier System (original method)		Arc Premier System (custom)		Arc Premier System (scale)		Arc Premier System Δ Area %	
	Mean area (%)	RSD (%)	Mean area (%)	RSD (%)	Mean area (%)	RSD (%)	custom	scale
1	4.92	4.07	3.17	1.58	3.30	2.73	1.76	-1.62
2	5.46	2.93	5.65	2.30	5.27	3.61	-0.19	-0.19
3	3.99	3.01	3.96	1.26	4.02	1.99	0.04	0.03
4	8.93	2.13	8.17	1.71	7.98	0.88	0.77	-0.95
5	8.27	4.47	8.31	1.20	8.13	1.97	-0.04	-0.14
6	14.33	2.37	15.62	1.28	15.15	2.11	-1.29	0.82
7	18.72	0.48	18.97	0.79	20.25	1.88	-0.25	1.53
8	8.00	3.38	8.11	1.97	8.70	2.87	-0.12	0.70
9	4.78	3.14	4.69	1.92	4.30	4.19	0.10	-0.48
10	9.63	1.25	10.10	1.98	9.85	1.22	-0.47	0.22
11	12.96	3.78	13.27	0.98	13.04	0.92	-0.30	0.08

Table 2: Evaluation of percentage peak area with migrated methods.

In addition to the Arc Premier System's ability to deliver consistent and reliable results, users have the added benefit of MaxPeak High-Performance Surfaces (HPS) Technology to reduce undesired analyte/surface interaction. Waters MaxPeak Premier columns with MaxPeak HPS Technology provide a robust and effective means to mitigate undesirable analyte/surface interaction. This is demonstrated in Figure 4 in terms of peak tailing factor for two acidic peptides, light chain (LC)-T13 and heavy chain (HC)-T38. As shown in Figure 4A, the LC-T13 peptide exhibited over a 60% reduction in peaks tailing (1.29 vs. 1.90) when using the XSelect™ Premier Peptide C_{18} Column compared to the conventional stainless-steel column. The improved performance observed when using the MaxPeak HPS Technology is critical when trying resolve impurities that are sensitive to analyte/surface adsorption. This is demonstrated for the HC-T38 peptide (PENNY peptide) which exhibited substantial tailing with the stainless-steel column that obscures the low-abundance impurity peaks eluting after it. When using the XSelect MaxPeak Premier Peptide C_{18} Column, these low-abundance peaks, identified to be deamidated forms of HC-T38, are both detectable and quantifiable. These results demonstrate the impact high-quality separations can have on peptide mapping assays and the value MaxPeak HPS Technology offers customers. The combination of system performance and system bio-inertness will lead to more robust assays that can be readily transferred and validated with increased confidence in data interpretation and less time spent at the bench.

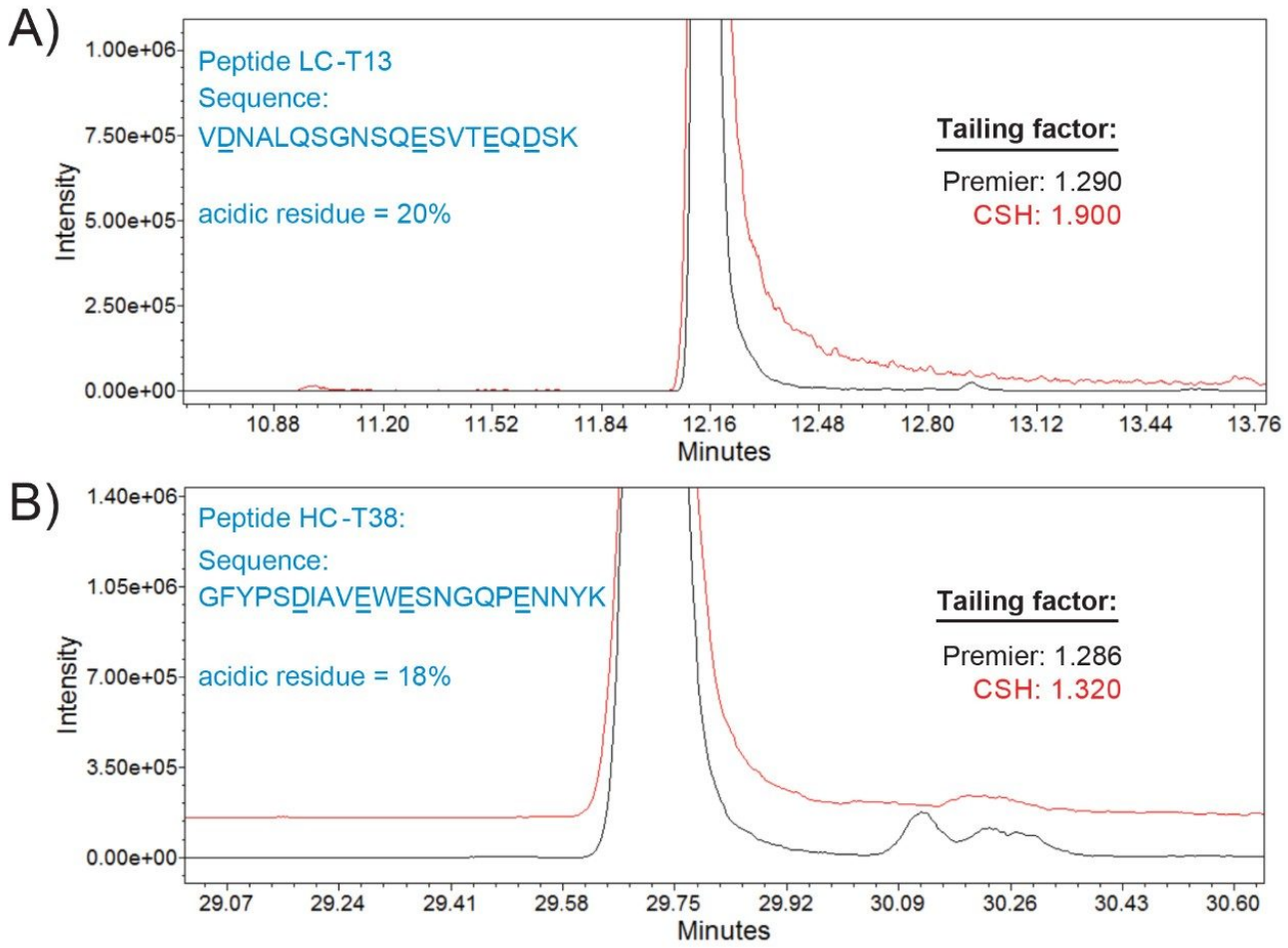

Figure 4: Evaluation of MaxPeak HPS Technology as a function of peak tailing for acidic amino acid containing peptide fragments A) LC-T13 and B) HC-T38.

Conclusion

It is critical that methods can be adapted to be run on new instrumentation while maintaining performance. In this application note, the Arc Premier System was able to reliably reproduce a peptide profile produced on an ACQUITY Premier System with peak area maintained within 5% and RRTs below 0.02 min indicating the separation performance and selectivity were conserved across methods. This is in part attributed to the Arc

Premier System's ability to deliver robust performance by reducing non-specific analyte/surface interaction via MaxPeak High Performance Surfaces as well as properly scaling methods through implementation of the Waters Columns Calculator. Collectively, these results demonstrate the Arc Premier System is well suited to support both LC/UV and LC/MS-based workflows in the development and manufacturing of biopharmaceuticals.

References

1. Robert E. Birdsall, Jacob Kellett, Ying-Qing Yu. Migration of an HILIC Method for Released Glycan Analysis from the ACQUITY UPLC H-Class PLUS Bio System to the Arc Premier System. Waters Application Note, 720007389. 2021 Sep.
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3. Pawel Bigos, Robert E. Birdsall, Ying-Qing Yu. Obtaining Equivalent IEX Chromatographic Performance Through Automated Method Scaling Using Waters™ Column Calculator. Waters Application Note, 720007807. 2022 Dec.

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