Application Note

# Obtaining Equivalent IEX Chromatographic Performance Through Automated Method Scaling Using Waters™ Column Calculator

Pawel Bigos, Robert E. Birdsall, Ying Qing Yu

Waters Corporation

## Abstract

As upstream workflows are developed and refined, associated analytical methods are eventually migrated downstream to support process development and routinely monitor product and process attributes associated with the active pharmaceutical ingredient. For biotherapeutics, monitoring of charge variants associated with monoclonal antibodies (mAbs) is critical to establish product stability and process consistency. In this respect, ensuring assay results remain consistent as methods are migrated downstream can save time spent on qualification and validation activities. In this application note, an ion-exchange chromatography (IEX) method for monoclonal antibodies (mAbs) was scaled using the Waters Column Calculator from an ACQUITY™ Premier System (UPLC™) to an Arc™ Premier System (UHPLC) representative of upstream and downstream LC configurations. Results from this study demonstrate that with appropriate scaling, methods can be migrated to downstream workflows, while retaining the chromatographic performance in terms of selectivity and peak area percent.

## Benefits

- ACQUITY Premier and Arc Premier systems deliver consistent results using pH or salt gradients
- BioResolve™ SCX mAb Columns are offered in a variety of dimensions to support IEX workflows across UPLC and UHPLC platforms
- BioResolve CX pH Buffer Concentrates enable reduced preparation and bench time to achieve robust and

reproducible cation-exchange separation

- The Waters Column Calculator offers straightforward method scaling with minimal user input

## Introduction

Pharmaceutical companies typically have a broad assortment of chromatography instruments deployed across laboratories to support development and manufacturing of biotherapeutics. As part of this dynamic, analytical methods are frequently developed and migrated across labs, ideally with consistent results. However, flow path differences across systems can impact system volume and dispersion, thereby affecting subsequent chromatographic results. Numerous parameters must be scaled appropriately to account for differences in column geometry and system dwell volume to maintain chromatographic performance regardless of separation technique (Figure 1). When scaling manually, these calculations take time to perform and are prone to error. Waters offers users a Column Calculator version 2.0 that has automated the method scaling process while requiring minimal user input.

To demonstrate this, the Waters Column Calculator was used to scale an IEX UPLC method developed on the ACQUITY Premier System and then migrated to an UHPLC method on the Arc Premier System. To enable optimized performance across systems, Waters offers the same BioResolve SCX mAb Column particle technology with 2.1 mm internal diameter columns for UPLC instruments and 4.6 mm columns for UHPLC instruments. With these column formats, scaled methods were migrated while preserving separation performance. Both pH and salt-based gradients were evaluated due to their prevalence in charge variant separation. In this work, percent peak area and relative retention time were used as metrics to demonstrate successful method migration of an upstream method to support downstream workflows.

## Experimental

Sodium chloride, MES monohydrate, MES salt, and caffeine was purchased from Sigma Aldrich. The Infliximab drug product Remicade™ was purchased from Amerisource Bergen and prepared (10 mg/mL) using sterile water

as per manufacturer's instructions and injected neat.

## ACQUITY Premier LC/UV Conditions[1]

| | |
|---|---|
| LC system: | ACQUITY Premier System (QSM-variant) |
| Detection: | ACQUITY TUV, FC=Ti 5 mm, λ=214 nm, 280 nm |
| Vials: | QuanRecovery™ Vials with MaxPeak™ High Performance Surfaces, (p/n: 186009186) |
| Column(s): | BioResolve SCX mAb Column, 3 µm, 2.1 mm x 100 mm (p/n: 186009056) |
| Column temperature: | 40 °C |
| Sample temperature: | 10 °C |
| Injection volume: | 1 µL |
| Flow rate: | 0.100 mL/min |
| Mobile phase A: | BioResolve CX pH Concentrate A (p/n: 186009063) or 20 mM MES buffer, pH 6.7 |
| Mobile phase B: | BioResolve CX pH Concentrate B (p/n: 186009064) or 20 mM MES buffer with 200 mM NaCl, pH 6.7 |
| Chromatography software: | Empower 3, FR4 |

## Arc Premier LC/UV Conditions

| | |
|---|---|
| LC system: | Arc Premier System (QSM-variant) |
| Detection: | 2489 UV/Vis Detector, 10 mm pathlength, λ=214 nm, 280 nm |
| Vials: | QuanRecovery Vials with MaxPeak High Performance Surfaces, (p/n: 186009186) |
| Column(s): | BioResolve SCX mAb Column, 3 µm, 4.6 mm x 100 mm (p/n: 186009060) |
| Column temperature: | 40 °C |
| Sample temperature: | 10 °C |
| Injection volume: | 4.8 µL |
| Gradient start - after injection: | 785 µL |
| Flow rate: | 0.480 mL/min |
| Mobile phase A: | BioResolve CX pH Concentrate A (p/n: 186009063) or 20 mM MES buffer, pH 6.7 |
| Mobile phase B: | BioResolve CX pH Concentrate B (p/n: 186009064) or 20 mM MES buffer with 200 mM NaCl, pH 6.7 |
| Chromatography software: | Empower 3, FR4 |

## ACQUITY Premier Gradient (BioResolve CX pH Concentrates)

| Time | Flow (mL/min) | %A | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| Initial | 0.100 | 81.5 | 18.5 | 0.0 | 0.0 | Initial |
| 45.00 | 0.100 | 61.0 | 39.0 | 0.0 | 0.0 | 6 |
| 46.00 | 0.100 | 10.0 | 90.0 | 0.0 | 0.0 | 6 |
| 49.00 | 0.100 | 10.0 | 90.0 | 0.0 | 0.0 | 6 |
| 50.00 | 0.100 | 81.5 | 18.5 | 0.0 | 0.0 | 6 |
| 70.00 | 0.100 | 81.5 | 18.5 | 0.0 | 0.0 | 6 |

## Arc Premier Gradient (BioResolve CX pH Concentrates)

| Time | Flow (mL/min) | %A | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| Initial | 0.480 | 81.5 | 18.5 | 0.0 | 0.0 | Initial |
| 45.00 | 0.480 | 61.0 | 39.0 | 0.0 | 0.0 | 6 |
| 46.00 | 0.480 | 10.0 | 90.0 | 0.0 | 0.0 | 6 |
| 49.00 | 0.480 | 10.0 | 90.0 | 0.0 | 0.0 | 6 |
| 50.00 | 0.480 | 81.5 | 18.5 | 0.0 | 0.0 | 6 |
| 70.00 | 0.480 | 81.5 | 18.5 | 0.0 | 0.0 | 6 |

### ACQUITY Premier Gradient (20 mM MES Buffer, pH 6.7)

| Time | Flow (mL/min) | %A | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| Initial | 0.100 | 91.0 | 9.0 | 0.0 | 0.0 | Initial |
| 38.00 | 0.100 | 72.0 | 28.0 | 0.0 | 0.0 | 6 |
| 39.00 | 0.100 | 0.0 | 100.0 | 0.0 | 0.0 | 6 |
| 44.00 | 0.100 | 0.0 | 100.0 | 0.0 | 0.0 | 6 |
| 45.00 | 0.100 | 91.0 | 9.0 | 0.0 | 0.0 | 6 |
| 70.00 | 0.100 | 91.0 | 9.0 | 0.0 | 0.0 | 6 |

### Arc Premier Gradient (20 mM MES Buffer, pH 6.7)

| Time | Flow (mL/min) | %A | %B | %C | %D | Curve |
|---|---|---|---|---|---|---|
| Initial | 0.480 | 91.0 | 9.0 | 0.0 | 0.0 | Initial |
| 38.00 | 0.480 | 72.0 | 28.0 | 0.0 | 0.0 | 6 |
| 39.00 | 0.480 | 0.0 | 100.0 | 0.0 | 0.0 | 6 |
| 44.00 | 0.480 | 0.0 | 100.0 | 0.0 | 0.0 | 6 |
| 45.00 | 0.480 | 91.0 | 9.0 | 0.0 | 0.0 | 6 |
| 70.00 | 0.480 | 91.0 | 9.0 | 0.0 | 0.0 | 6 |

## Results and Discussion

Throughout the life cycle of a drug product, from drug discovery to a marketed drug, analytical methods are migrated downstream from characterization labs to manufacturing and quality control workflows. Ideally, the downstream method should provide comparable results to historical data generated from earlier developed

analytical methods. In downstream workflows, instruments supporting manufacturing and quality control are engineered to be more forgiving toward method conditions and tend to have larger internal diameters for tubing and columns for increased robustness. As shown in Figure 1, several parameters must be considered to account for these differences to successfully migrate a method downstream. The Waters Column Calculator is able to automate these calculations with minimal user input. From the parameters shown, only dwell volume offset is dependent on the individual system and must be determined experimentally.

| Parameter | Calculation |
|---|---|
| **Column efficiency** | $L_1/dp_1 = L_2/dp_2$ |
| **Column volume** | $V = \pi r^2 l$ |
| **Injection volume** | $Vi_2 = Vi_1 \; x \; (V_2/V_1)$ |
| **Flow rate** | $F_2 = F_1 x \left(\frac{dp_1}{D_1^2}\right) \Big/ \left(\frac{dp_2}{D_2^2}\right)$ |
| **Run time** | $t_2 = t_1 x \left(\frac{V_2}{F_2}\right) \Big/ \left(\frac{V_1}{F_1}\right)$ |
| **Dwell volume offset** | $DV = Vd_1 \; - (Vd_2 \; x \; \frac{V_1}{V_2})$ |
| **Column volume of Gradient segment** | $GS = (FT)/\mathrm{V}$ |

| Variable definition | | |
|---|---|---|
| $L$ | = | column length |
| $dp$ | = | particle diameter |
| $V$ | = | column volume |
| $Vd$ | = | system dwell volume |
| $D$ | = | column diameter |
| $r$ | = | column radius |
| $l$ | = | column length |
| $Vi$ | = | injection volume |
| $F$ | = | flow rate |
| $T$ | = | segment duration |
| $t$ | = | run duration |
| $GS$ | = | column volume of the gradient segment |

*Figure 1. Parameters that need to be considered to appropriately scale methods.*

In this study, system dwell volume was determined following the conditions listed in Figure 2 for the ACQUITY Premier System and Arc Premier System.[2] The dwell volume results as well as the column dimensions and ACQUITY Premier systems gradient conditions were entered into the Waters Column Calculator (Figure 3). Due to the differences in column geometry between Premier systems, the Column Calculator appropriately scaled the flow rate and injection volume to maintain identical column loading and column volumes. Based on the dwell volume difference between systems, the Column Calculator recommended an isocratic hold of 785 µL for the migrated Arc Premier System to match the retention times observed from the ACQUITY Premier System. These

scaled conditions were entered as method parameters in Figure 3.

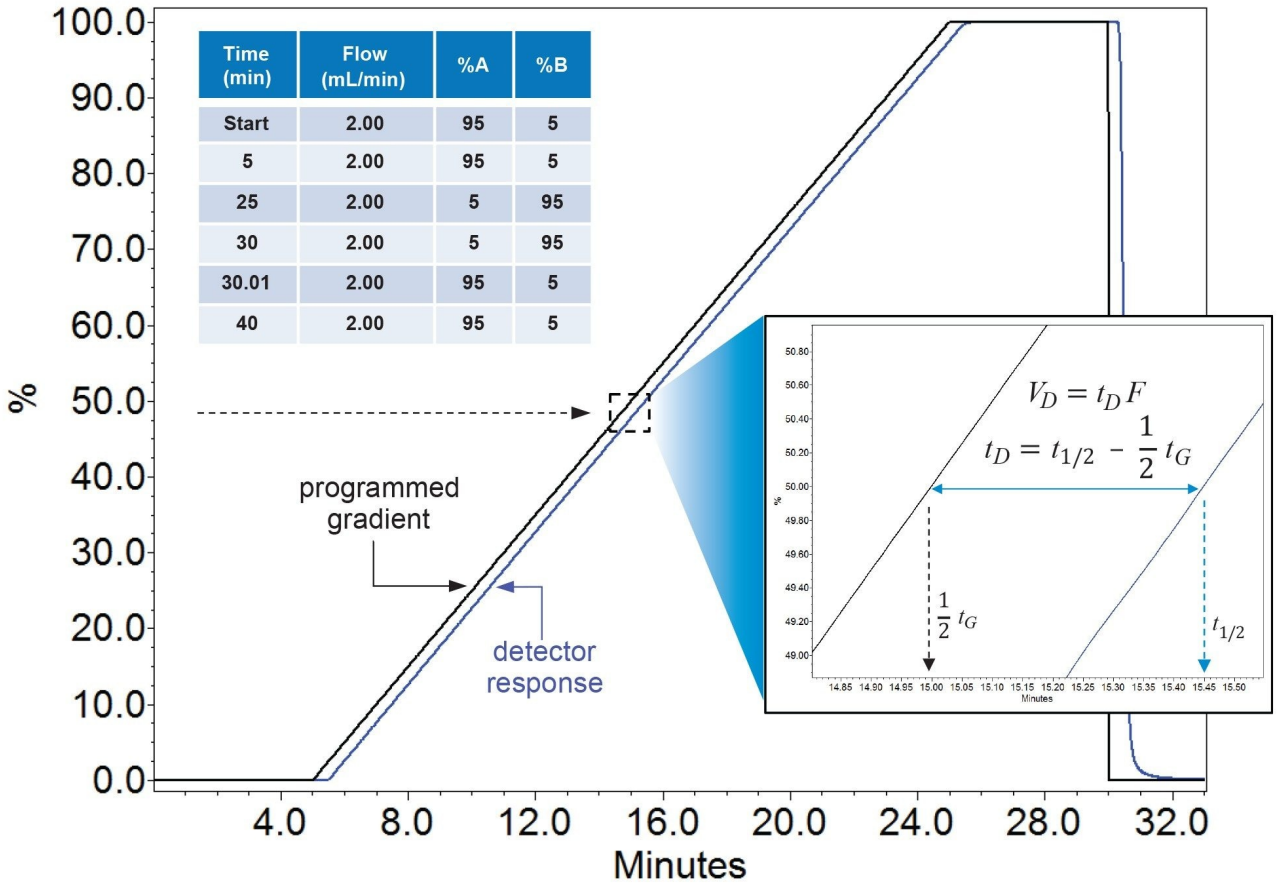



*Figure 2. Measuring dwell volume by calculating the difference between the detector response and programmed gradient. Mobile phase A: Water, Mobile phase B: 10mg/L caffeine in water, wavelength: 273 nm.*

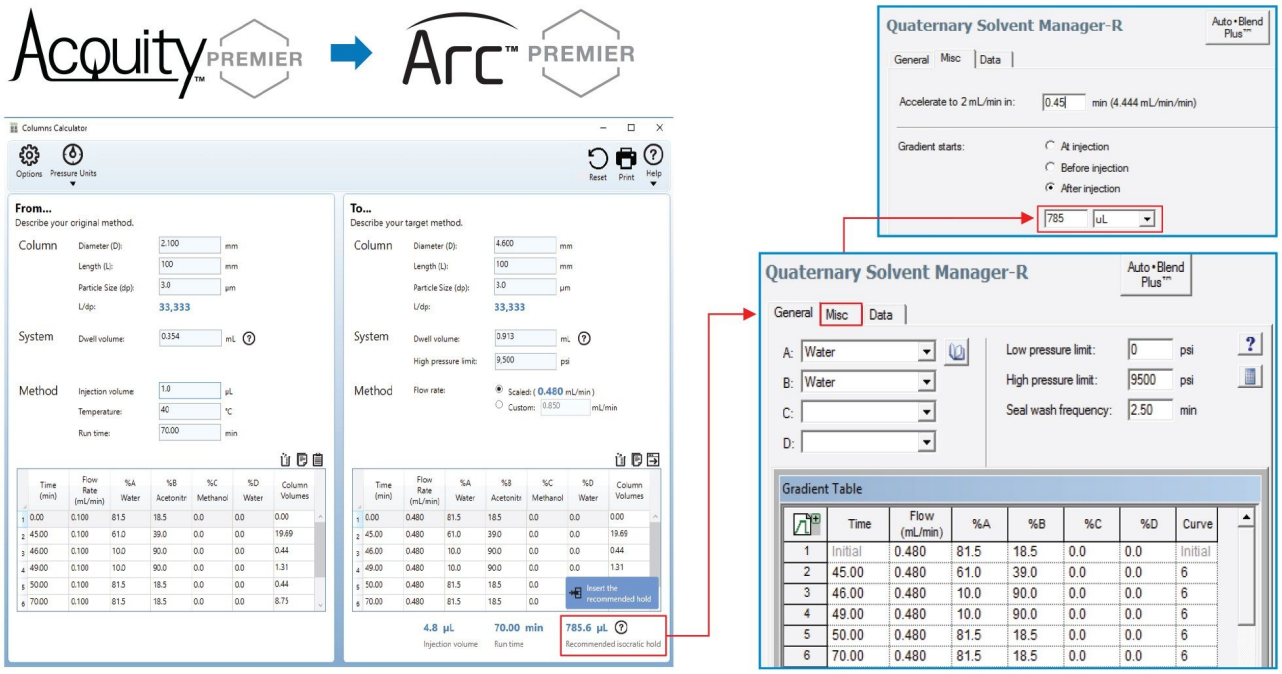



*Figure 3. Incorporating scaled parameters and system dwell volume differences into the instrument method.*

The method migrated in this work was an IEX-based method using BioResolve CX pH Concentrates and BioResolve SCX mAb Columns. These BioResolve pH Concentrates are engineered to deliver reproducible linear pH gradients for optimal separation of charge variants when using IEX-based columns such as the BioResolve SCX mAb Column. While maintaining the same resolving power (length/particle size ratio), two BioResolve SCX mAb Columns with different internal diameters were used in the original and scaled method. Remicade, a mAb-based therapy, was analyzed using the original method as well as the scaled method with/without dwell volume adjustments (Figure 4). In both cases the %RSD for retention time is below 1% for five replicate injections demonstrating the reproducibility of the BioResolve Column technology. However, the reader can see from the middle chromatogram, when the isocratic hold is not utilized, the overall profile has shifted by approximately two minutes. While acceptable in terms of maintaining peak resolution, in a regulated lab environment where retention times for analytical methods are specified, matching the chromatographic profile is important for adequate method migration. When the isocratic hold is used (Figure 4, bottom chromatogram), the chromatographic profiles align closely from the original method.

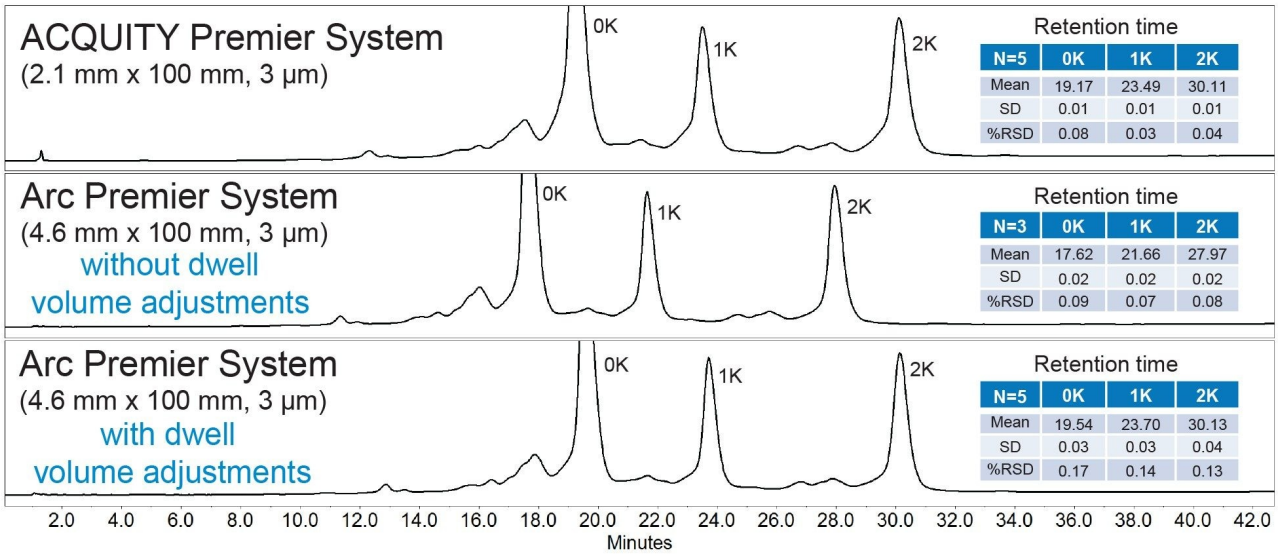
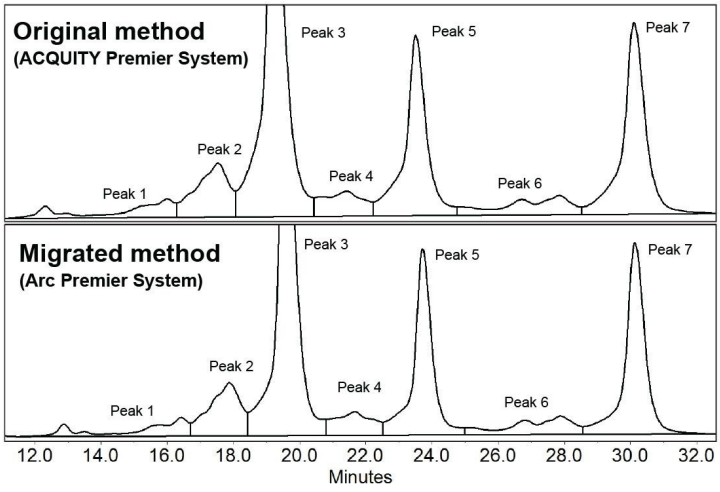



*Figure 4. Evaluation of retention time differences with migrated methods when dwell volume differences are and are not accounted for.*

The relative retention time and relative percent peak area were calculated for five replicate injections to evaluate the scaled methods ability in terms of reproducibility and chromatographic performance. As shown in Figure 5, the absolute difference in relative retention time was below 0.03 for the scaled method and the relative percent peak area was within ~0.5% of the original method. In addition, a conventional salt gradient was scaled to evaluate the ability of the BioResolve columns to preserve chromatographic performance using more traditional mobile phases to demonstrate broader applicability. The scaled method maintained comparable selectivity and peak area minimizing the need for further optimization for both gradients (Figure 5). These results highlight the robust performance of both instruments and the ability for the Column Calculator to properly scale IEX methods.




**Relative retention time**

| N=5 | BioResolve CX pH concentrates | | | MES salt buffer | | |
|---|---|---|---|---|---|---|
| | ACQUITY Premier | Arc Premier | Absolute difference | ACQUITY Premier | Arc Premier | Absolute difference |
| Peak 1 | 0.834 | 0.841 | 0.007 | 0.823 | 0.808 | 0.015 |
| Peak 2 | 0.914 | 0.915 | 0.001 | 0.890 | 0.877 | 0.013 |
| Peak 3 | 1.000 | 1.000 | 0.000 | 1.000 | 1.000 | 0.000 |
| Peak 4 | 1.117 | 1.108 | 0.009 | 1.106 | 1.094 | 0.012 |
| Peak 5 | 1.226 | 1.213 | 0.013 | 1.219 | 1.219 | 0.000 |
| Peak 6 | 1.453 | 1.427 | 0.026 | 1.454 | 1.450 | 0.004 |
| Peak 7 | 1.571 | 1.542 | 0.029 | 1.561 | 1.570 | 0.009 |

**Relative % peak area**

| N=5 | BioResolve CX pH concentrates | | | MES Salt Buffer | | |
|---|---|---|---|---|---|---|
| | ACQUITY Premier | Arc Premier | Absolute difference | ACQUITY Premier | Arc Premier | Absolute difference |
| Peak 1 | 4.408 | 4.486 | 0.078 | 4.258 | 4.644 | 0.386 |
| Peak 2 | 8.106 | 8.628 | 0.522 | 7.480 | 7.558 | 0.078 |
| Peak 3 | 38.410 | 38.316 | 0.094 | 38.324 | 38.448 | 0.124 |
| Peak 4 | 4.704 | 4.660 | 0.044 | 5.670 | 5.714 | 0.044 |
| Peak 5 | 18.362 | 18.288 | 0.074 | 17.652 | 17.530 | 0.122 |
| Peak 6 | 5.396 | 5.350 | 0.046 | 5.594 | 5.546 | 0.048 |
| Peak 7 | 20.608 | 20.264 | 0.344 | 21.020 | 20.560 | 0.460 |

*Figure 5. Relative retention time and relative percent peak area comparison between the original and migrated method using pH and salt-based gradients.*

## Conclusion

The Waters Column Calculator is a valuable tool for quickly scaling methods by automating the calculation of operating parameters that provide equivalent chromatographic performance. This application note demonstrated that the calculated method conditions on a scaled UHPLC system replicated the original UPLC methods performance. When comparing between systems, the relative retention time of Remicade had an absolute difference below 0.03 and relative peak areas were within 0.5% of the original method for both pH and salt-based gradients. When appropriately scaled, IEX methods can be migrated between systems seamlessly and expected to generate robust performance.

## References

1. Birdsall RE, Koshel BM, Yu YQ. Accelerating Charge Variant Analysis of Biotherapeutics with the BioAccord™

System. Waters Application Note. 2022. 720007706.

2. Hong P, McConville PR. Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer. Waters Application Note. 2018. 720005723. <https://www.waters.com/webassets/cms/library/docs/720005723en.pdf>

## Featured Products

Arc Premier System <https://www.waters.com/waters/nav.htm?cid=135083359>

ACQUITY Premier System <https://www.waters.com/waters/nav.htm?cid=135077739>

2489 UV/Visible (UV/Vis) Detector for Purification and Modular LC Systems <https://www.waters.com/134721513>

Empower Chromatography Data System <https://www.waters.com/10190669>

BioResolve CX pH Concentrate A <https://www.waters.com/nextgen/global/shop/standards--reagents/186009063-bioresolve-cx-ph-concentrate-a.html>

BioResolve CX pH Concentrate B <https://www.waters.com/nextgen/global/shop/standards--reagents/186009064-bioresolve-cx-ph-concentrate-b.html>

BioResolve SCX mAb Column, 3 µm, 4.6 mm x 100 mm, 1/pk <https://www.waters.com/nextgen/global/shop/columns/186009060-bioresolve-scx-mab-column-3--m-46-mm-x-100-mm-1-pk.html>

BioResolve SCX mAb Column, 3µm, 2.1 mm X 100 mm, 1/pk <https://www.waters.com/nextgen/global/shop/columns/186009056-bioresolve-scx-mab-column-3-m-21-mm-x-100-mm-1-pk.html>

QuanRecovery with MaxPeak HPS 12 x 32 mm Screw Neck Vial, 300 µL, 100/pk <https://www.waters.com/nextgen/global/shop/vials-containers--collection-plates/186009186-quanrecovery-with-maxpeak-hps-12-x-32-mm-screw-neck-vial-300--l-.html>

Waters Column Calculator <https://www.waters.com/waters/support.htm?lid=134891632&type=DWNL>

720007807, December 2022