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From UHPLC to HPLC: Key Considerations for Method Scaling

Paula Hong, Margaret Maziarz, and Stephanie Harden
Waters Corporation, Milford, MA, USA

INTRODUCTION

Validated analytical methods ensure the quality, safety, and efficacy of pharmaceutical products. Methods are developed under strict regulatory frameworks and are expected to deliver consistent, reproducible results throughout the product lifecycle. As laboratories evolve, whether through the adoption of new instrumentation, changes in manufacturing strategy, or the need to transfer[1] methods across global sites, these validated methods often require adaptation.

Moreover, as drug development pipelines accelerate and regulatory expectations tighten, the ability to adapt by scaling chromatographic methods efficiently across different platforms becomes increasingly vital. Scaling is distinct from method development or transfer. Method scaling in liquid chromatography (LC) is the adaptation of analytical procedures, such as adapting to different column formats or instrument configurations. These changes may be driven by differences in instrument characteristics, the need to increase sample throughput, reduce solvent usage, increase sensitivity or specificity, or improve overall operational efficiency.

The introduction of ultra-performance liquid chromatography (UPLC™) in 2004 revolutionized liquid chromatography[2] by setting a new benchmark for analytical performance, enabling faster, more efficient separations with higher resolution and reduced solvent consumption. Since then, the breadth of commercially available ultra-high performance liquid chromatography (UHPLC) systems has increased significantly. However, high-performance liquid chromatography (HPLC) systems with larger particle-sized columns (typically 3-µm or more) and operating at lower pressures, are still widely used in laboratories globally. Methods developed on UHPLC systems often need to be 'scaled up' for use on these more traditional technologies. The scaled methods must produce comparable performance and results to the original method to assure product quality and compliance with regulatory requirements.

This white paper describes considerations and approaches for scaling gradient methods from sub-2-µm columns to larger particles for use with HPLC instruments. Numerous factors that can impact the success of method scaling are discussed, including the principles of scaling column chemistry, impact of key system characteristics including, but not limited to, dwell volume and extra-column dispersion, the impact of method settings and specific system configurations. It also highlights how Waters™ technologies, including scalable column families, advanced LC systems, and compliance-ready software, can support successful method scaling while maintaining data integrity and regulatory alignment.

REGULATORY CONSIDERATIONS FOR SCALING ANALYTICAL METHODS

Scaling of chromatographic methods, whether in-house developed or pharmacopeial, must be undertaken with scientific rigor and in alignment with current regulatory expectations. A risk-based approach, supported by robust change control and continuous performance monitoring, ensures long-term compliance and analytical reliability.

For pharmacopeial methods, the United States Pharmacopeia (USP) General Chapter <621>[3] provides clear guidance on allowable method adjustments. As of the December 2022 revision, modifications to gradient conditions, column dimensions, and particle types are permitted — including transitions from totally porous particles (TPPs) to superficially porous particles (SPPs) — provided the L/dp ratio remains within −25% to +50% of the original method. Adjustments to flow rate, gradient time, and injection volume must be proportional to column geometry, and gradient profiles should be scaled to preserve slope and retention characteristics. However, USP <621> emphasizes that system suitability criteria must continue to be met, and that cumulative changes may necessitate method verification (USP <1226>[4]) or full revalidation (USP <1225>[5]) depending on their potential impact.

For in-house methods, adjustments fall under the broader framework of analytical procedure lifecycle management, as described in ICH Q14.[6] This guideline supports risk-based decision-making by encouraging a thorough understanding of method parameters, their criticality, and their relationship to method performance. This allows for justified, flexible post-approval changes when backed by appropriate scientific data. ICH Q12[7] further enables the implementation of change management protocols (PACMPs) to streamline regulatory submissions for anticipated method modifications.

Regardless of method origin, any adjustment must be governed by a formal change control process that includes impact assessment, scientific justification, revalidation or verification as appropriate, and documentation aligned with CGMP expectations. Moreover, continuous monitoring of method performance is vital to detect potential drifts or issues post-adjustment, supporting a state of control throughout the method lifecycle.

By integrating USP, ICH, and CGMP principles, organizations can make method scaling decisions that are not only scientifically sound but also compliant with evolving global regulatory expectations, preserving analytical integrity and product quality over time.

A STEP-BY-STEP APPROACH TO SCALING ANALYTICAL METHODS

Scaling methods from UHPLC to HPLC is not simply a matter of resizing columns or adjusting flow rates. It requires a nuanced understanding of chromatographic principles and a systematic strategy to ensure that changes to method parameters are made in a controlled, scientifically justified manner. This helps maintain method performance, reproducibility, and compliance with regulatory expectations. A systematic approach to method scaling involves a number of steps, including knowledge gathering and risk assessment, identification of an appropriate column, determining the parameters to adjust, testing the method, analyzing the results, and monitoring the method over time (Figure 1).

Within this white paper, these steps will be described using examples showing the impact of scaling methods from lower dispersion UHPLC systems to higher dispersion HPLC systems.

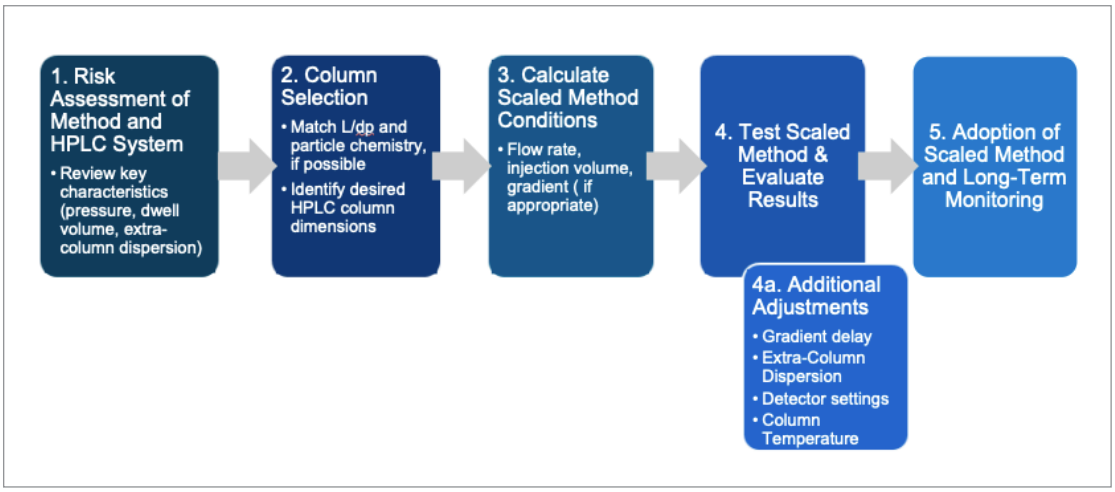

Figure 1. Systematic workflow for scaling an analytical method.

1. RISK ASSESS THE INSTRUMENT AND METHOD

The first step of scaling a UHPLC method to HPLC is to understand and risk-assess both the method and the receiver HPLC system. It is essential to identify and fully understand any attributes that may impact the ability to achieve comparable separation. When adapting a method to a different system, it is important to compare both hardware specifications and method settings. These settings may not be explicitly covered in standard guidance documents, but are particularly critical, and should be reviewed to ensure compatibility with the receiver system.

Information can be gathered and risk-assessed in a suitable format, such as a table or a fishbone diagram. Figure 2 illustrates the fishbone diagram used for the risk assessment of scaling a hydrophilic interaction chromatography (HILIC) method for the analysis of ribavirin from UHPLC to HPLC.[8] The mechanism of separation and the high organic starting conditions make HILIC methods uniquely sensitive to both method and instrument characteristics, including sample diluent mismatch, needle washes, mobile phase pH and column equilibration.[9] In this example (Figure 2), key method and instrument characteristics were reviewed and sample diluent, gradient table, needle wash, and the use of ammonium hydroxide were identified as aspects that could be of concern when moving the method to an HPLC system.

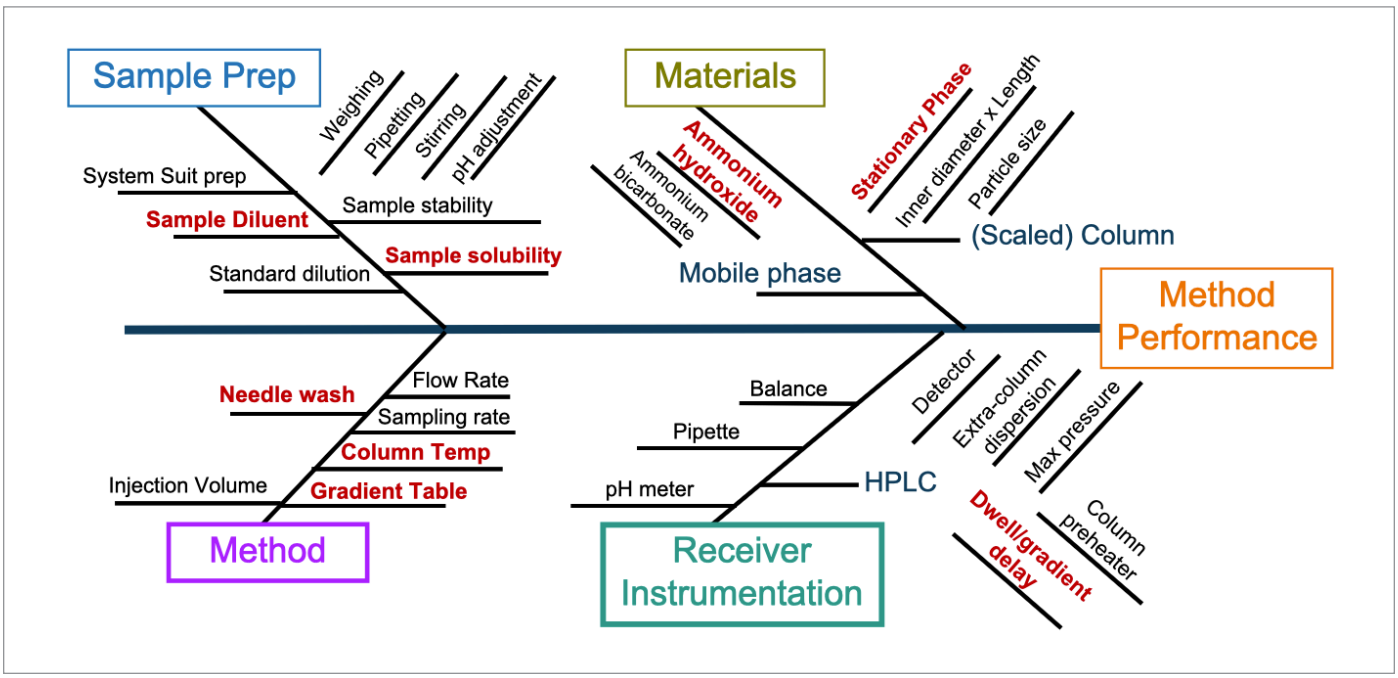

Figure 2. Fishbone risk assessment of scaling HILIC separation of ribavirin from UHPLC to HPLC.

Reviewing Instrument Characteristics

The influence of system pressure, dwell volume, and extra-column dispersion on HPLC method performance can vary significantly depending on method-specific parameters such as column dimensions, particle size, and analyte properties. Methods with narrow peaks, fast gradients, or closely eluting compounds are more sensitive to these system differences, while later eluting or wider peaks in isocratic methods may be more tolerant. Gradient methods can be especially affected by dwell volume, and UHPLC methods using small particles are more vulnerable to dispersion and pressure limitations when scaled to HPLC systems. Table 1 illustrates the key characteristics compiled for both the originator system and receiver systems in the ribavirin HILIC method scaling example.[8]

System Class	System	Maximum Operating Pressure	Dwell Volume*** (mL)	Extra-Column Dispersion* µL (4σ)
UHPLC	ACQUITY UPLC H-Class Plus	15,000	~ 0.475	~ 8-10
HPLC	Alliance HPLC	5,000	~ 1.15	~ 40 to 60
HPLC	Arc HPLC	10,000	~ 1.35	~ 40 to 55
HPLC	Alliance iS HPLC	12,000	~ 1.64	~ 26

Measurements taken on a single configuration or single system
**Dispersion measurements are dependent on specific configuration. With/without preheat \ CH vs CM\ PDA vs TUV etc.*
Table 1. Key instrument characteristics of HPLC and UHPLC systems.

Maximum System Operating Pressure

The maximum system operating pressure is related to instrument design. UHPLC systems have higher maximum operating pressures to accommodate columns packed with smaller, sub-2-µm, particles. HPLC systems typically have lower maximum pressures, however, even within a range of systems the operating pressure range may vary (Table 1). If a system has a lower maximum operating pressure than required for scaled conditions, then flow rate adjustment can be made.

System Dwell Volume or Gradient Delay

The dwell volume or gradient delay of a chromatographic system is the total volume between the point of mobile phase mixing and the inlet of the column.[10] For gradient methods, determination of the dwell volume is important,[11,12] since it can impact the elution time of analytes and the equilibration of a method. While not described under method conditions, the gradient delay acts as an isocratic hold at the beginning of the gradient. This delay is held constant throughout the gradient and can be particularly impactful at the end of the gradient or at the point at which initial conditions are reached. Excessive gradient delays can impact or reduce the re-equilibration of the column. To address any issues with gradient delay, the instrument mixer can be changed, and/or adjustments for dwell volume can be made. This will be discussed further in the method scaling section.

Extra-Column Volume or System Dispersion

Extra-column volume, often referred to as dispersion, is the volume from the point of injection to the detector flow cell, excluding the column.[11] The extra-column effects are related to the physical components of the system, including the internal diameter of the tubing, valves, connectors to the column, fittings, detector flow cells, *etc.*

When scaling chromatographic methods, it's important to consider the relationship between extra-column volume and column volume, as this can significantly impact separation performance. Column volume refers to the internal volume of the column, determined by its length and internal diameter excluding the volume of the packing material, and influences peak volume, efficiency, and retention behavior. In UHPLC systems, where columns are typically shorter and packed with sub-2-µm particles, peak widths are narrow and low volume. As a result, even modest extra-column volumes, such as those from tubing, detector cells, or injector loops, can cause peak broadening and loss of resolution, making low-dispersion system design critical.

In contrast, HPLC systems generally use larger volumn columns with larger particle sizes, resulting in broader peak volumes. These larger peaks are less sensitive to extra-column effects, making HPLC methods inherently more robust in this regard. However, if the extra-column volume becomes disproportionately large relative to the column volume, resolution loss can still occur. Therefore, while the impact of extra-column volume is more pronounced in UHPLC, it should not be overlooked in HPLC, especially when scaling methods across platforms with different system configurations.[12]

2. SELECT AN APPROPRIATE COLUMN

When scaling chromatographic methods from UHPLC to HPLC, column selection is guided by both the parameters of the original method and the performance characteristics of the target instrument. Preserving selectivity and reproducibility requires careful matching of stationary phase chemistry. As stated by the USP, beyond aligning the ligand, "the other physiochemical characteristics of the stationary phase…must be similar".[3] This is particularly important because columns classified under the same USP category (*e.g.*, L1 or C_{18}) can still demonstrate markedly different selectivity for identical analytes.[13] Therefore, thorough evaluation of column chemistry is essential during method transfer.[14]

In HPLC systems, columns with larger internal diameters (typically 3.0 or 4.6 mm) and larger particle sizes (such as 3.5- or 5-µm) are commonly used. Larger IDs help mitigate the effects of extra-column dispersion,[15] which can degrade separation quality, while larger particles reduce backpressure, making the method compatible with lower-pressure HPLC systems. Additionally, consistency in base particle type and stationary phase is important, as variations in packing material can significantly affect chromatographic behavior. If the UHPLC column has an HPLC equivalent in both column particle technology and ligand, risk in column selection can be reduced.

Once the stationary phase is selected, column length (L) and internal diameter (ID) must be considered. These dimensions influence resolution, analysis time, and separation efficiency. A key metric in scaling is the L/dp ratio (column length divided by particle diameter), which correlates with column efficiency and resolution. As mentioned earlier, USP <621> guidelines[3] specify that, for monograph methods, adjustments to L/dp should remain within -25% to +50% of the original method to maintain consistent chromatographic behavior. For example, a UHPLC method using a 50-mm column with 1.7-µm particles yields an L/dp of approximately 29,400. To scale this method appropriately, HPLC column options should aim to match this ratio (Table 2A). As UHPLC column length increases (*e.g.*, to 100 mm), the corresponding HPLC column must also be lengthened (*e.g.*, to 150 mm or more; Table 2B) to maintain a consistent L/dp ratio. However, practical constraints such as instrument column compartment size may limit available options, making it necessary to balance method requirements with system compatibility.

A. Scaling from 1.7 µm and 50 mm column

Method	Particle size (dp) µm	Length (L) mm	L/dp
Original	1.7	50	29,412
Target	2.5	75	30,000
	3.5	100	28,571
	5.0	150	30,000

B. Scaling from 1.7 µm and 100 mm column

Method	Particle size (dp) µm	Length (L) mm	L/dp
Original	1.7	100	58,824
Target	2.5	150	60,000
	3.5	150	42,857
	5.0	300	60,000

Table 2. Example of column dimensions and particle size when scaling from a 1.7-µm column, which is 50 mm in length (A), versus 100 mm in length (B).

3. CALCULATE SCALED METHOD CONDITIONS

Once the column dimensions and particle size are identified, the method can be scaled. Specifically, the flow rate, injection volume, and gradient times are scaled geometrically to maintain the separation quality and performance.[16-18] The adjusted flow rate ensures the same linear velocity is maintained from the original method to the scaled method. The modified flow rate is based on the internal diameter of the columns, the particle size of the columns, and the original flow rate and can be calculated using the following formula:

$$F_2 = F_1 \times [(dc_2^2 \times dp_1) / (dc_1^2 \times dp_2)]$$

Where F_1 and F_2 are the flow rates (mL/min) for the original and scaled method, dc_1 and dc_2 are the column internal diameters (mm) and dp_1 and dp_2 are the particle sizes (µm) of the original and scaled methods.[3]

The injection volume must be scaled to maintain the column volume to injection volume ratio, and to maintain sensitivity, linearity, and resolution. Injection volume is scaled according to column volume with the following formula:

$$V_{inj2} = V_{inj1} (L_2 dc_2^2) / (L_1 dc_1^2)$$

Where V_{inj1} and V_{inj2} are the injection volumes for the original and scaled methods, and L_1 and L_2 are the column lengths and dc_1 and dc_2 are the column internal diameters (mm) for the original and scaled methods, respectively.

Lastly, the gradient step must be kept constant in terms of column volumes. To do this, the time of each gradient step must be calculated for the original method and then preserved for the scaled method. The time of each step is calculated as follows:

$$t_{G2} = t_{G1} \times (F_1 / F_2) [(L_2 \times dc_2^2) / (L_1 \times dc_1^2)]$$

Where all values are as previously described and t_{G1} and t_{G2} are the gradient time in the original and scaled method, respectively.

To facilitate this process, the Waters Columns Calculator[19-21] can be used to perform method scaling calculations based on both column dimensions and system parameters, as illustrated in Figure 3. Based on the principles described above, it compensates for dwell volume differences by adjusting injection volume and introducing an isocratic hold, ensuring consistent gradient start times. The calculator also maintains constant column volume during gradient steps and provides expected system pressure estimates. The calculator also allows for flow rate adjustments, within the ±50% as permitted.[3]

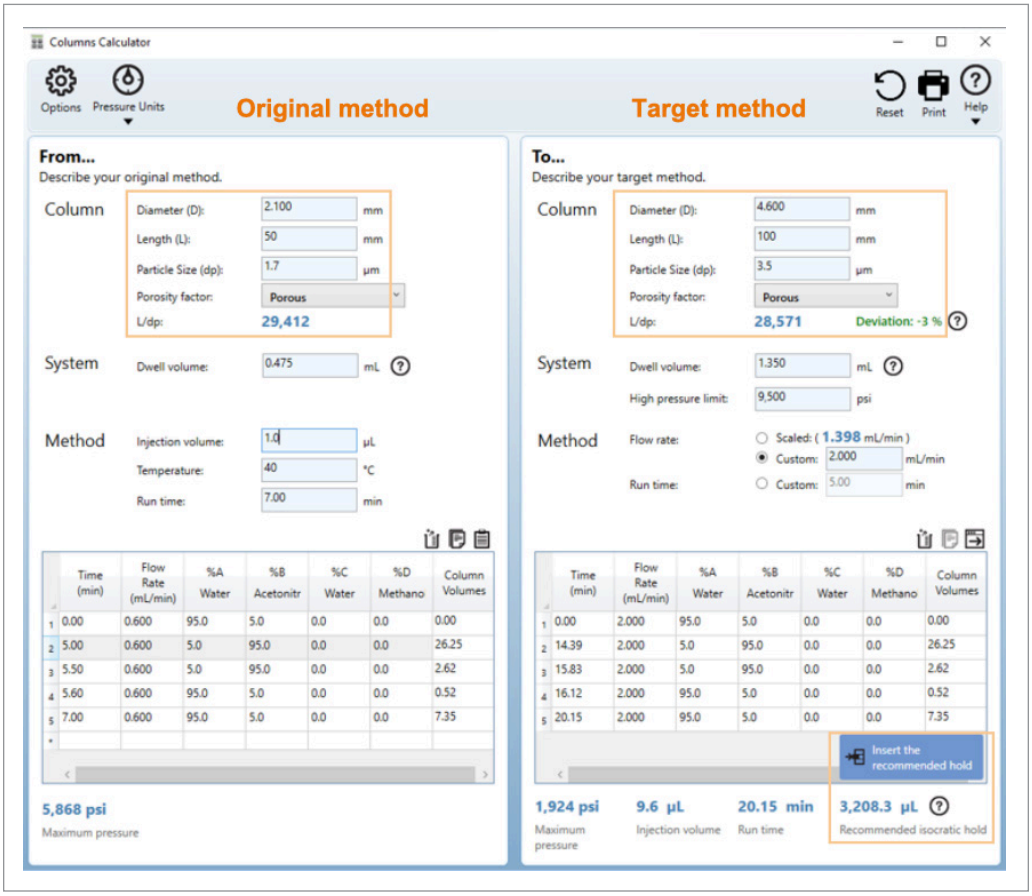

Figure 3. Gradient method scaling using the Waters Columns Calculator. The tool adjusts for dwell volume differences with an isocratic hold, scales gradient steps to maintain column volume, and estimates system pressure.

Impact of Gradient Delay or Dwell Volume When Scaling Methods

When scaling methods, it is important to keep the delay constant in terms of number of column volumes.[16,17] Many UHPLC systems designed for use with sub-2-µm particle-size columns have smaller dwell volumes ranging from ~0.04 to 0.7 mL, while HPLC systems typically have larger delay volumes of >1.0 mL (Table 1). If values are known or measured on both systems, the dwell volume can be considered for adjustment of gradient start at injection. A general rule, if values are not known, is to use a gradient delay adjustment of approximately one column volume.

While general recommendations are to use an isocratic step at the start of a gradient, adjustment of dwell volume can also be performed. This adjustment can be performed based on elution time of peaks or using the Waters Columns Calculator, which accounts for the differences in system dwell volume and column void volumes. In the example given (Figure 3), the measured dwell volumes of the original and the target systems are entered: 0.475 mL for the original UHPLC system and 1.35 mL for the target HPLC system. As discussed earlier, you can think of the gradient delay in terms of column volumes (Dcv). Thus, the calculations work out as:

Dcv = D/cv where D is the dwell volume (mL) and cv = column volume (mL).

$$Dcv_1\left(Originator\ \ system\right) = \frac{D}{cv} = \frac{0.475}{0.1143} = 4.165\ \ cv$$

$$Dcv_2\left(Receiver\ \ system\right) = \frac{D}{cv} = \frac{1.35}{1.097} = 1.231\ \ cv$$

Based on these values, to use the 4.6 x 100 mm column on an HPLC system, you should increase the initial hold to mimic the gradient delay by approximately 2.925 cv or 3.21 mL.

Adjustment of an initial hold for a gradient method can be performed several ways. The initial hold of the gradient can be changed, or, in some method editors, the gradient start can be adjusted relative to the injection (Figure 4). The latter allows adjustment in volume (µL) or time (minutes), allowing you to ensure the same delay in column volumes in the scaled method.

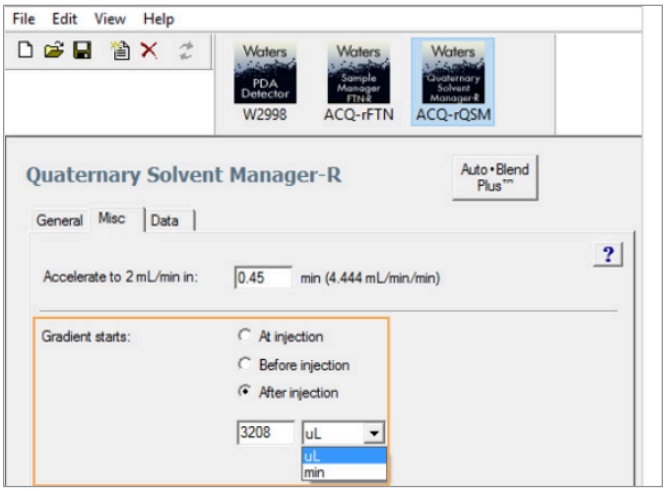

Figure 4. Adjustment of gradient delay for method scaling using the Gradient SmartStart function in Waters Empower™ Software, with a Waters ACQUITY™ Arc™ System instrument method.

4. TEST SCALED METHOD CONDITIONS AND REVIEW RESULTS

Following the risk assessment, instrument and column selection, and the adjustment of the method, an analysis can be performed on the receiving HPLC system. Where errors occur, due to over-pressurization of the system or parameters that are not within receiving system's operating range, additional adjustments to flow rate and run time may be required to ensure compatibility.

It is critical to thoroughly review the data acquired from the scaled method to ensure system suitability criteria are met. This includes verifying parameters such as retention time consistency, peak resolution, tailing factors, and baseline stability. Meeting system suitability ensures that the method is performing reliably on the receiving system and that the analytical results are valid. If any criteria are not met, further method adjustments, such as changes to flow rate, temperature, or injection volume, may be necessary.

Additional adjustments

Even after appropriately scaling column dimensions, flow rate, and injection volume, further adjustments may be needed to meet system suitability. Regulatory guidance such as ICH Q2(R2)[22] highlights detector settings and temperature control as critical variables affecting method performance. Differences in detector response time, sampling rate, or effective column temperature can alter retention, resolution, or baseline stability, making them important factors to assess during method adjustment and robustness evaluation.

Detector settings

The detector sampling rate (or sampling frequency) refers to the number of data points collected per second during a chromatographic run.[23] This rate directly affects how well peaks are defined, influencing signal quality, peak width, and baseline noise. A sufficient sampling rate, typically around 20-30 data points per peak, is essential to ensure accurate integration, especially for trace-level impurity analysis. However, sampling too frequently can increase baseline noise and data file size, impacting sensitivity. Therefore, selecting an appropriate rate is a balance between achieving sufficient data points for reliable quantitation and minimizing unnecessary noise.[24]

Low-dispersion systems with sub-2-µm columns generate narrow peaks, generally requiring higher sampling rates. In contrast, conventional HPLC peaks are broader, so lower sampling rates can still provide accurate results. To demonstrate the effects, a study based on the USP monograph for diclazuril organic impurities[24] was performed (Figure 5). The UPLC separation using a 2.1 x 50 mm, 1.8-µm column produced narrow peaks with acceptable sensitivity and resolution for the critical pair (diclazuril/diclazuril ketone) using a sampling rate of 20 Hz (Figure 5A). When scaling to an HPLC system and column (4.6 x 100 mm, 3.5-µm column), the same sampling rate delivered efficient separation with acceptable USP signal-to-noise and USP resolution (Figure 5B) with the wider HPLC peaks. However, lowering the HPLC method sampling rate to 5 Hz on the HPLC system improved USP signal-to-noise 2x without negatively impacting resolution (Figure 5C), demonstrating the optimum lower sampling rates for HPLC columns and corresponding peak widths.

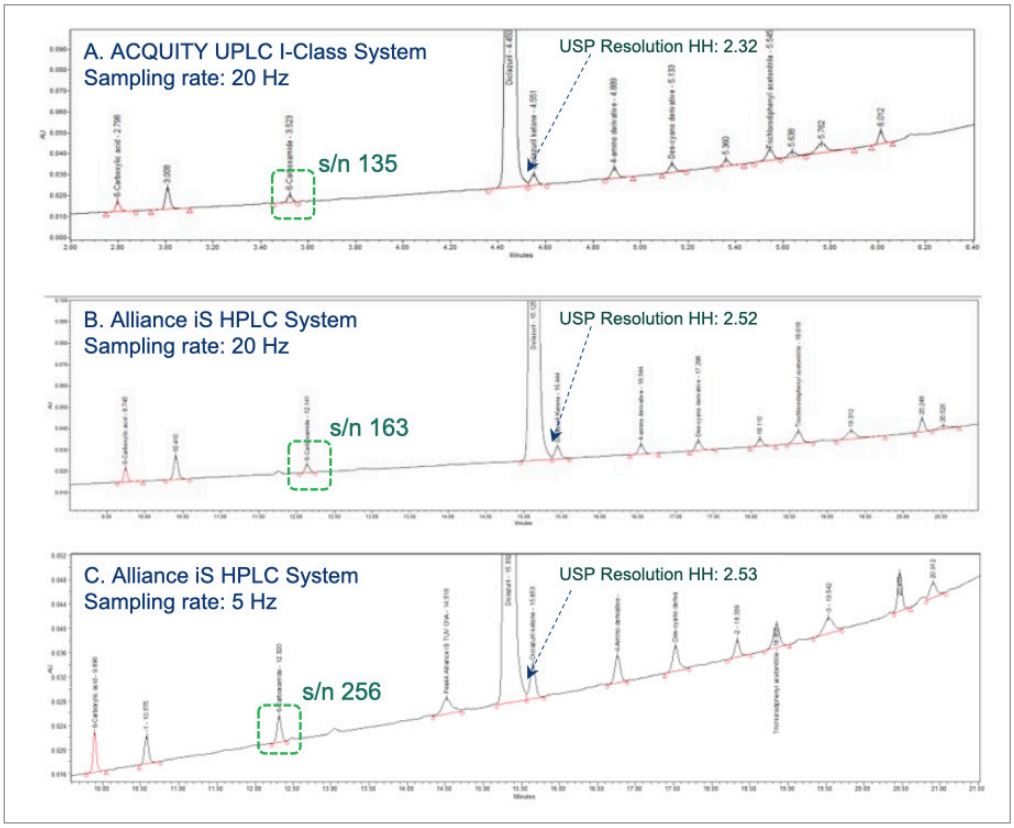

Figure 5. Example of the detector sampling rate impact on separation efficiency. Data acquired on an ACQUITY UPLC I-Class PLUS System with 20 Hz (A) and Alliance iS HPLC System with 20 Hz (B) and 5 Hz (C).

Temperature effects

Another factor that can impact chromatographic separations in method adjustments is the impact of column temperature. Column heaters or thermostatted column compartments of LC systems can vary in performance. LC systems may use open-air convection or static heating column compartments, and the configuration of the column compartment may accommodate a single column or multiple columns, employing valves to switch between columns. Lastly, mobile phase preheater design may vary, either the heat can be distributed via contact with a heated surface (passive) or via electronic control (active). These differences can affect retention, selectivity, and peak shape, especially in temperature-sensitive methods.[24]

An example of the importance of controlling column temperature in both originator and scaled method is shown in Figure 6. An impurity method originally analyzed on an ACQUITY UPLC H-Class PLUS System equipped with an active pre-heater required an elevated temperature of 45 °C (Figure 6A). The method was scaled to HPLC and analyzed on an Arc HPLC System, without and with passive preheating. Without passive preheating, band broadening and peak distortion was observed due to thermal mismatch (Figure 6B), the temperature difference between the mobile phase and the column when the mobile phase enters the column.

With the addition of passive pre-heating, however, the Arc HPLC System produced comparable separation to that of the original method (Figure 6C). Thus, when scaling methods across LC systems, the type of column compartment and nature of mobile phase preheating should be taken into consideration to retain the integrity of the chromatographic separation.

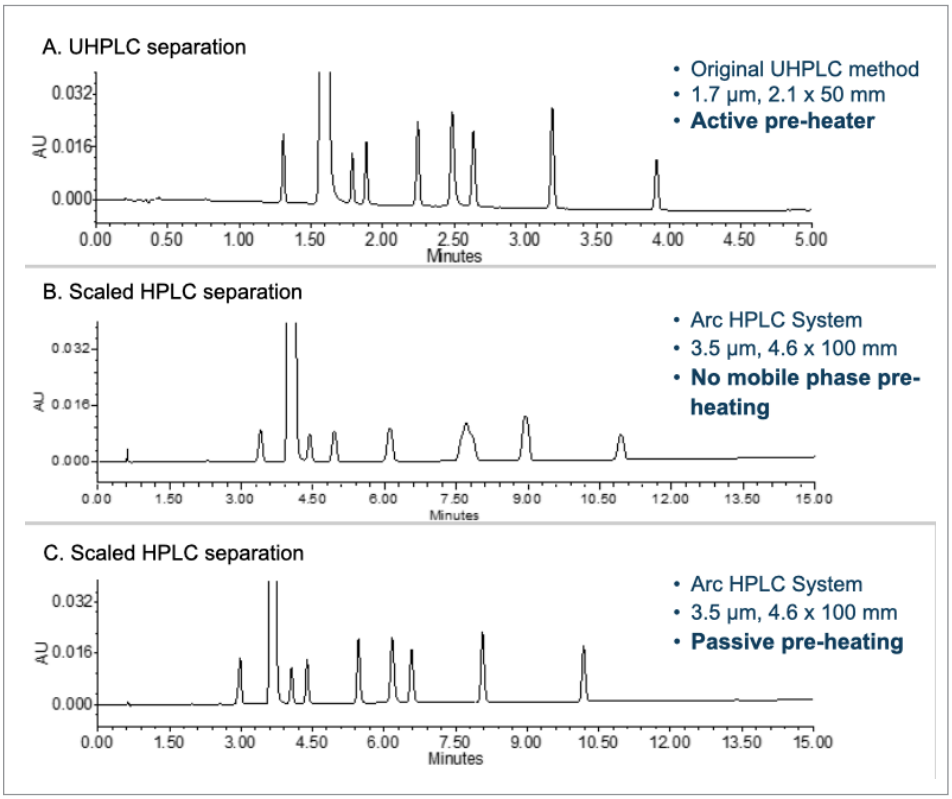

Figure 6. Effect of temperature control when scaling chromatographic methods. UHPLC separation (A) and scaled HPLC separation without (B) and with passive pre-heating (C).

Long-term monitoring

Ongoing monitoring after post-approval method changes is critical to ensure continued method performance, reliability, and regulatory compliance.[25] The ICH Q14 and USP <1220> guidance emphasize the importance of post-change oversight. Monitoring strategies may include system suitability testing, trending of analytical data, and periodic performance reviews. These practices help detect variability early, support continuous improvement, and maintain confidence in the method's ability to ensure product quality, safety, and efficacy throughout its lifecycle.

CONCLUSION

Scaling UHPLC methods to HPLC platforms is often necessary as laboratories adapt to evolving technologies, workflows, and regulatory demands. Within this white paper, we have outlined a systematic, science-based approach to method scaling that balances performance, compliance, and practicality. Key factors, including column selection, system characteristics, gradient delay, and detector settings, must be carefully evaluated and adjusted to preserve method integrity. Tools such as the Waters Columns Calculator streamline this process by automating critical calculations and compensating for system differences like dwell volume. Equally important is long-term monitoring post-implementation. Routine performance checks and trending of analytical data ensure the scaled method continues to perform reliably throughout its lifecycle.

With a thorough understanding of chromatographic principles and the right tools and technologies, method scaling can be achieved efficiently, enabling labs to maximize instrument capabilities while maintaining the quality and consistency demanded by modern pharmaceutical analysis.

EXAMPLE: SCALING A HILIC SEPARATION OF RIBAVIRIN

In this example, a HILIC method for the analysis of active pharmaceutical ingredient ribavirin and related compounds was scaled from a 1.7-µm (2.1 x 50 mm) column to a 3.5-µm (4.6 x 100 mm) column with equivalent chemistry (Table 3) and migrated from an ACQUITY UPLC H-Class PLUS System to both the Arc HPLC System and the Alliance™ iS HPLC System (Figure 7). Both system and method characteristics were risk assessed and adjustments, including compensation of the gradient delay, were made to ensure compatibility with the receiving columns and systems.

Parameter	Conditions for 1.7 µm column	Scaled conditions for 3.5 µm column
Systems	ACQUITY™ UPLC™ H-Class Plus System, ACQUITY™ PDA, active pre-heating	• Arc™ HPLC System, 2998 PDA, passive pre-heater • Alliance™ iS HPLC System with TUV Detector, passive pre-heating
Column	ACQUITY™ UPLC™ BEH™ Amide, 2.1 x 50 mm, 1.7 µm (P/N: 186004800)	XBridge™ BEH™ Amide, 4.6 x 100 mm, 3.5 µm (P/N: 186004868)
L/dp	29,412	28,571
Flow rate	0.5 mL/min	2.0 mL/min
Gradient	Step / Time / %A / %B / %C / Curve 1 / Initial / 90.0 / 5.0 / 5.0 / Initial 2 / 1.50 / 90.0 / 5.0 / 5.0 / 6 3 / 3.00 / 30.0 / 65.0 / 5.0 / 6 4 / 4.00 / 30.0 / 65.0 / 5.0 / 6 5 / 4.10 / 90.0 / 5.0 / 5.0 / 6 6 / 7.00 / 90.0 / 5.0 / 5.0 / 6	Step / Time / %A / %B / %C / Curve 1 / Initial / 90.0 / 5.0 / 5.0 / Initial 2 / 3.60 / 90.0 / 5.0 / 5.0 / 6 3 / 7.20 / 30.0 / 65.0 / 5.0 / 6 4 / 9.60 / 30.0 / 65.0 / 5.0 / 6 5 / 9.70 / 90.0 / 5.0 / 5.0 / 6 6 / 14.00 / 90.0 / 5.0 / 5.0 / 6
Inj. volume	1.0 µL	9.6 µL

Table 3. Geometrically scaled method conditions calculated using the Waters Columns Calculator.

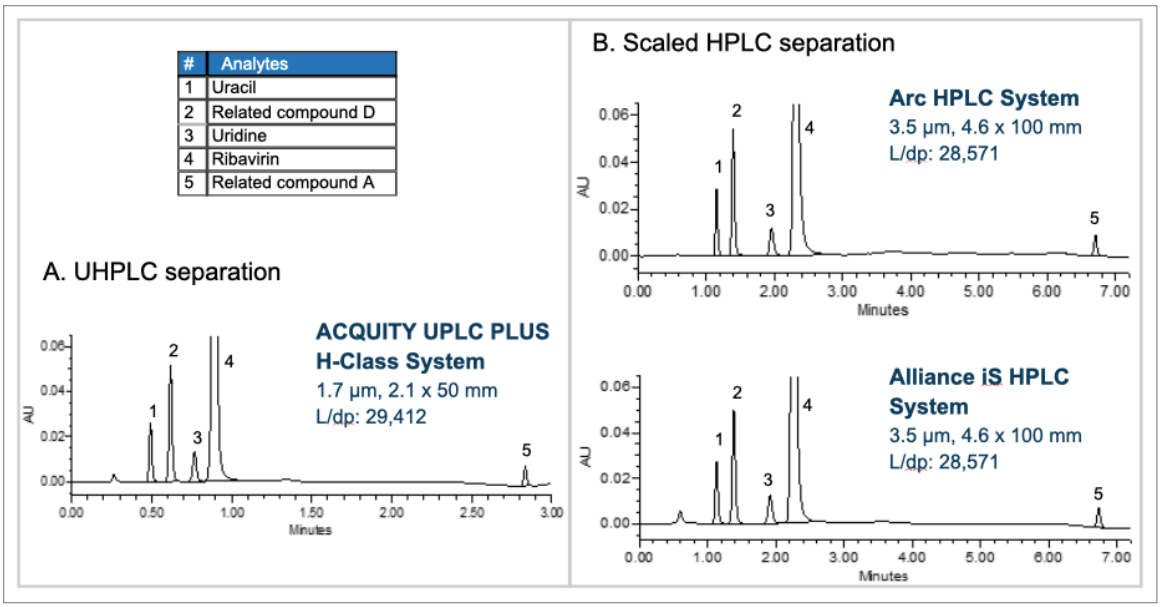

Figure 7. The original UHPLC (A) and final scaled HPLC (B) HILIC separation of ribavirin and related compounds.

Review of the results (Table 4A) demonstrated that the scaled methods met the required acceptance criteria. No significant loss of resolution was observed, and area precision was within the expected variability of the method. Furthermore, the retentivity (k*) of the separation was preserved (Table 4B).

A. System Suitability

System Suitability Criteria	Compounds	ACQUITY UPLC H-Class PLUS System 1.7 µm column	Arc HPLC System 3.5 µm column	Alliance iS HPLC System 3.5 µm column
USP Resolution: NLT 2.0	■ Uracil and related compound D	3.3	4.2	3.1
	■ Uridine and ribavirin	2.7	3.2	2.9
Retention times: RSD of NMT 2.0%	Ribavirin	0.10	0.47	0.05
Peak areas: RSD of NMT 2.0%	Ribavirin	0.22	0.36	0.39

B. Retentivity Factor (k*)

Compounds	ACQUITY UPLC H-Class PLUS System 1.7 µm column	Arc HPLC System 3.5 µm column	Alliance iS HPLC System 3.5 µm column
Uracil	0.9	1.0	1.0
Rel comp-D	1.3	1.5	1.4
Uridine	2.0	2.4	2.4
Ribavirin	2.5	3.0	3.0
Rel comp-A	9.5	10.9	10.8

Table 4. Results for ribavirin and related compounds method scaling from UHPLC to HPLC. System suitability (A) and retentivity factor (B). NLT: not less than, NMT: not more than, RSD: relative standard deviation.

References

1. Chung, Z. Modernizing Method Transfer in QC Labs: Comparative Testing, Continuous Monitoring, and Digital Enablement. Waters Whitepaper, 720008975, 2025.
2. Turner, J.E., Jenkins, K.M. The Evolution of Ultra High-Performance Liquid Chromatography: Expanding the Future of Separation Technologies. Chromatography Today, 2018.
3. USP General Chapter <621> Chromatography https://www.usp.org/harmonization-standards/pdg/excipients/chromatography.
4. USP General Chapter <1226>: Verification of Compendial Procedures https://doi.usp.org/USPNF/USPNF_M870_03_01.html.
5. USP General Chapter <1225>: Validation of Compendial Procedures https://doi.usp.org/USPNF/USPNF_M99945_04_01.html.
6. ICH Q14: Analytical Procedure Development https://database.ich.org/sites/default/files/ICH_Q14_Guideline_2023_1116_1.pdf.
7. ICH Q12: Technical and regulatory considerations for pharmaceutical product lifecycle management https://database.ich.org/sites/default/files/Q12_Guideline_Step4_2019_1119.pdf.
8. Maziarz, M. Scaling and Migration of a Hydrophilic Interaction Liquid Chromatography (HILIC) Method for Related Compounds of Ribavirin to Modern HPLC Systems, Waters Application Note, 720008255, 2024.
9. Grumbach, E.S., Fountain, K.J. Comprehensive Guide to HILIC: Hydrophilic Interaction Chromatography, Waters Corporation, https://www.waters.com/nextgen/global/shop/education/715002531-comprehensive-guide-to-hilic-hydrophilic-interaction-chromatogra.html.
10. Dolan, J.W. Dwell Volume Revisited. LCGC North America-05-01-2006 Volume24 Issue 5, Pages: 458–466, https://www.chromatographyonline.com/view/dwell-volume-revisited-0.
11. Hong, H., McConville, P.R. Dwell Volume and Extra-Column Volume: What Are They and How Do They Impact Method Transfer? Waters White Paper, 720005723, 2018.
12. How do I determine system dwell volume – WKB50707. Waters Knowledge Base article. Accessed 27-Feb-2023, https://support.waters.com/KB_Chem/Other/WKB50707_How_do_I_determine_system_dwell_volume.
13. Berthelette, K.D., DeLoffi, M., Haynes, K. Examining Column Selectivity Differences Across Various L1 Designation Columns for Paracetamol EP Impurities Monograph. Waters Application Note, 720008395, 2024.
14. Walter, T.H., Gilar, M., et al. Stationary Phase Selectivity in HILIC: The Importance of Ionic Interactions, Waters poster, PSTR135123446.
15. Dolan, J.W. Column Diameter, Linear Velocity, and Column Efficiency. LCGC North America, 28:2, 2010.
16. Dolan, J.W. LC Method Scaling, Part II: Gradient Separations. LCGC, 32:3, pages: 188-193, 2014.
17. Dlugasch, A.B., Simeone, J., McConville, P.R. Gradient Method Scaling for Life Cycle Management of a USP Impurities Method. Waters Application Note 720006577, 2019.
18. Berthelette, K.D., Turner, J.E., Kalwood, J., Haynes, K. Faster, Simpler Method Scaling Across Particles Sizes Using the Waters Columns Calculator Compared to Manual Calculation Workflow. Waters Application Note, 720007887, 2023.
19. Maziarz, M. Scaling and Migration of a Method for Naphazoline Hydrochloride, Pheniramine Maleate and Associated Related Substances to an ACQUITY™ Arc™ System. Waters Application Note, 720008189, 2024.
20. Berthelette, K.D., Turner, J.E., Kalwood, J., Haynes, K. Faster, Simpler Method Scaling Across Particles Sizes Using the Waters Columns Calculator Compared to Manual Calculation Workflow. Waters Application Note, 720007887, 2023.
21. ICH Q2(R2): Validation of Analytical Procedures https://database.ich.org/sites/default/files/ICH_Q2(R2)_Guideline_2023_1130.pdf.
22. Amini, K., Gauthier, L., Simeone, J., Hong, P. Optimizing Detector Settings for Method Scaling — Meeting System Suitability Criteria (Poster M1030-06-40). AAPS PharmSci 360 Meeting, Waters Corporation, 2023.
23. Empower Tip: Importance of Sampling Rate and Time Constant for Chromatography, https://help.waters.com/help/en/product-support/empower-chromatography-data-system-support/empower-tips/tip-207.html.
24. Li, Z., Hong, P. McConville, P.R. The Importance of Column Compartment Thermostatting and Preheating for Temperature Sensitive Separations in Liquid Chromatography, Waters Application Note, 720007137, 2021.
25. Navigating USP <1221> What the New Guidelines Mean for Analytical Laboratories. Waters White Paper, 720008976, 2025.

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