Case study | 000566

Liquid chromatography

Method transfer onto the Thermo Scientific Vanquish HPLC/UHPLC platform: A CDMO perspective

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Introduction

Themo Fisher Scientific Pharma Services Group (PSG, also known as Patheon) provides industry-leading pharma service solutions in drug development, clinical trials logistics, and commercial manufacturing. With more than 55 locations worldwide, expertise in chemical and biotherapeutic molecule drug substances, and drug products across the product lifecycle, the Pharma Services Group is well regarded as a leader in pharma services. Addressing such a wide range of drug substances and products, as well as demanding timelines and operating in a cGMP environment, PSG needs to be flexible and have streamlined processes while continuing to be compliant with regulatory agency requirements.

To meet the growing demands for a variety of projects, PSG has recently embarked on a technology refresh program to replace aging analytical equipment with a more modern liquid chromatography platform. The chosen technology needed to be compatible with their existing IT infrastructure (Waters™ Empower™ 3 Chromatography Data Software), be suitable for the analysis of both chemical and biologic molecules, and have both HPLC and UHPLC performance for compatibility with legacy HPLC methods as well as more modern UHPLC assays. Meeting all the requirements, the Thermo Scientific™ Vanquish™ UHPLC platform was installed across the PSG network. The transition to this platform presented some hesitancy for analysts familiar with other technologies as well as clients who had developed their methods on other vendor's LC systems. However, greater flexibility, ease-of-use, enhanced robustness, and serviceability significantly outweighed these challenges. By combining current regulatory guidance, industry best practices, and understanding of the Vanquish platform, successful method transfers to the Vanquish platform were accomplished.

Guidance on how to approach method transfer from a CDMO perspective

A case study (CS000565)[1] providing insights on how to overcome liquid chromatography method transfer complements this case study and provides guidance based on regulatory requirements and analytical quality by design (AQbD). To summarize, there is currently no formal guidance from the ICH on method development/modernization from an AQbD standpoint, and there are limited modifications that are allowed for compendial methods. However, a change in column packing (maintaining the same USP column code), the duration of an initial isocratic hold (when prescribed), and/or the gradient delay volume (also known as dwell volume) are allowed when transferring a method. Adjusting gradient delay volume is the preferred approach to transfer and modernize an existing method as it can lead to quickly developed robust methods without modifying the system from its intended purpose—in other words, not leading to method validation and, more importantly, instrument qualification.

Gradient delay volume is defined as the volume between the point of mobile phase mixing and the column entry. More precisely, it is the combined volume contributed by pumping system, gradient mixer, tubing between the pump and the injector, injector, and tubing between the injector and the column. It should be noted that this only impacts gradient methods and that the column is not affected by gradient changes until the solvents have traveled the length of the gradient delay volume.

Systems with larger gradient delay volumes will have longer times between mobile phase condition changes (and injections) and the observation of chromatographic impact (detector response). In other words, larger gradient delay volume will lead to later eluting peaks in gradient separations and can modify the elution pattern due to the longer isocratic holdup at the beginning.

Modern UHPLC systems like the Thermo Scientific™ Vanquish™ Flex and Thermo Scientific™ Vanquish™ Horizon UHPLC systems are designed to have smaller gradient delay volume than traditional HPLCs. Chromatographic peaks will thus elute earlier on these systems.

There are two approaches to compensate for gradient volume difference between different liquid chromatography systems. The first approach involves method adaption of the isocratic hold to simulate the same chromatography between systems with different gradient delay volumes. The second approach is to have hardware modifications, such as mixer and sample loop exchange, to emulate gradient delay and mixing behavior. With proper documentation and a simple verification test, these hardware modifications will typically not require instrument requalification as the instrument still meets its intended purpose. Additionally, a tunable gradient delay volume solution, such as the one available on the Thermo Scientific™ Vanquish™ HPLC platform, will enable gradient delay volume adjustments without the need to replace instrument hardware.

Vanquish analytical liquid chromatography portfolio

There are three main analytical scale liquid chromatography systems in the Vanquish portfolio. The entry line Vanquish Core system, which is designed to be similar to traditional HPLC systems, the mid-tier Vanquish Flex system, designed for application flexibility, and the top of the line Vanquish Horizon system, which provides ultimate performance for the most demanding laboratory. In the PSG analytical development laboratory, the Vanquish Flex system meets their needs. Out of the box, it is biocompatible, allowing analysis of small and large molecules, and is compatible with both HPLC and UHPLC methods.

Table 1. The Vanquish portfolio

	Vanquish Core HPLC systems	Vanquish Flex UHPLC systems	Vanquish Horizon UHPLC systems
Specialty	Dependable routine HPLC analysis	Reliable UHPLC and flexible method development	Unrivaled high-end UHPLC performance
Backpressure limit (bar)	700	1,000	1,500
Dwell volume			
Biocompatibility		✓	✓

Determining gradient delay volume

Gradient delay volume contributors include the pumping system, autosampler volume, and associated connective tubing and mixers. It should be noted that the pump is typically the largest contributor to gradient delay volume for quaternary systems. Gradient delay volume can be measured as follows.

1. Run a gradient from 0% solvent B to 100% solvent B (inject solvent A or 0 µL) (Table 2).
   - Option 1 (preferred): solvent A: water | solvent B: 10 mg/mL caffeine in water
   - Option 2: solvent A: MeOH | solvent B: 10 mg/mL acetophenone in MeOH

   Adjust the isocratic hold times at the beginning and end of the gradient as needed to capture the system delay.

2. Calculate the gradient delay volume using the following equation:

   Gradient delay volume = FR × (T50 – (0.5 × TG))

   where:

   FR = Flow rate in mL/min

   T50 = Time of 50% response

   TG = Time of Gradient (exclude hold times)

   For example, using values from Figure 1:

   0.5 mL/min × (10.5 min – (0.5 × 20 min))

   0.5 mL/min × 0.5 min

   = 0.25 mL or 250 µL

Table 2. Experimental gradient delay volume calculation

Time (min)	%MPA	%MPB
0	100	0
20	0	100
22	0	100

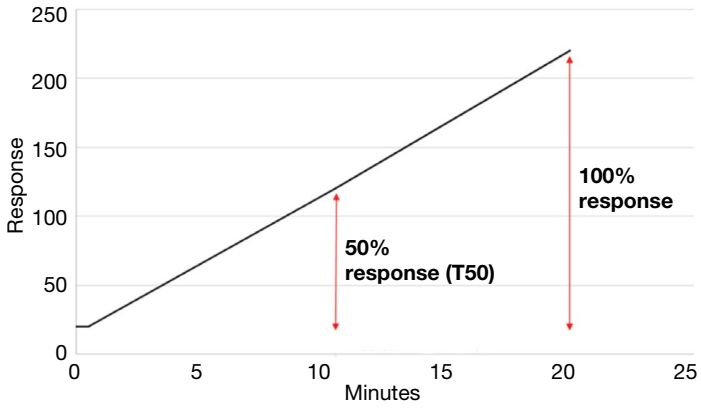

Figure 1. Experimental gradient delay volume calculation

Alternatively, gradient delay volumes (GDVs) can be calculated if volumes of each pump and autosampler components are known. For convenience, the tables below indicate typical gradient delay volumes for Vanquish systems of various configurations. As a reminder, the gradient delay volume is calculated as the sum of the autosampler volume, invariable volume of system tubing (capillaries), and the volume of mixer in the pump.

Table 3. Vanquish Horizon gradient delay volume

	Delivery state Nominal sample loop volume = 25 µL			**Optional Nominal sample loop volume = 10 µL**			**Optional Nominal sample loop volume = 100 µL**	
	Minimum	Default	Maximum	Minimum	Default	Maximum	Minimum	Default
Sampler GDV	110 µL	135 µL	210 µL	83 µL	93 µL	183 µL	190 µL	290 µL
Invariable system tubing	5 µL (based on delivery state)							
Mixer + inline filter volume	35 µL (based on delivery state)							
Minimum system GDV	150 µL			123 µL			230 µL	
Factory set system GDV		**175 µL**			**133 µL**			**330 µL**
Maximum system GDV			250 µL			223 µL		

Table 4. Vanquish Flex Binary gradient delay volume

	Delivery state Nominal sample loop volume = 25 µL			Optional Nominal sample loop volume = 10 µL			Optional Nominal sample loop volume = 100 µL	
	Minimum	Default	Maximum	Minimum	Default	Maximum	Minimum	Default
Sampler GDV	110 µL	135 µL	210 µL	83 µL	93 µL	183 µL	190 µL	290 µL
Invariable system tubing	5 µL (based on delivery state)							
Mixer + inline filter volume	200 µL (based on delivery state)							
Minimum system GDV	315 µL			288 µL			395 µL	
Factory set system GDV		**340 µL**			**289 µL**			**495 µL**
Maximum system GDV			415 µL			388 µL		

Table 5. Vanquish Flex Quaternary gradient delay volume

	Delivery state Nominal sample loop volume = 25 µL			Optional Nominal sample loop volume = 10 µL			Optional Nominal sample loop volume = 100 µL	
	Minimum	Default	Maximum	Minimum	Default	Maximum	Minimum	Default
Sampler GDV	110 µL	135 µL	210 µL	83 µL	93 µL	183 µL	190 µL	290 µL
Invariable system tubing	5 µL (based on delivery state)							
Pump GDV volume	679 µL (default)							
Minimum system GDV	794 µL			767 µL			874 µL	
Factory set system GDV		**819 µL**			**777 µL**			**974 µL**
Maximum system GDV			894 µL			867 µL		

Table 6. Vanquish Core Quaternary gradient delay volume

	Delivery state Nominal sample loop volume = 100 µL			Optional Nominal sample loop volume = 10 µL			Optional Nominal sample loop volume = 250 µL		
	Minimum	Default	Maximum	Minimum	Default	Maximum	Minimum	Default	Maximum
Sampler GDV	230 µL	255 µL	480 µL	123 µL	148 µL	373 µL	425 µL	450 µL	675 µL
Invariable system tubing	25 µL (based on delivery state)								
Pump GDV volume	679 µL (default)								
Minimum system GDV	934 µL			827 µL			1129 µL		
Factory set system GDV		**959 µL**			**852 µL**			**1154 µL**	
Maximum system GDV			1184 µL			1077 µL			1379 µL

Modifying the Vanquish UHPLC system to mimic other systems

By knowing the differences in gradient delay volume of the origin and target liquid chromatography system, transferring the method is straightforward. The analyst must look only at which parts of the system need to be changed to match the originator system gradient delay volume. Standard gradient delay volumes of various liquid chromatography systems are shown in Table 7.

Table 7. Standard gradient delay volumes of various liquid chromatography systems

Liquid chromatography system	Gradient delay volume
Waters™ Alliance™	1,100–1,400 µL
Waters™ ACQUITY™ Arc™	760–1,100 µL
Waters™ ACQUITY™ H-Class with 100 µL mixer	380 µL
Waters ACQUITY™ H-Class with 250 µL mixer	530 µL
Agilent™ 1100	1,200–1,300 µL (pressure dependent)
AgilentTM 1260 Infinity I[a]	1,100 µL (pressure dependent)
AgilentTM 1260 Infinity II low volume configuration[b]	290 µL (pressure dependent)
Agilent™ 1290 Infinity™ II	300 µL (pressure dependent)

[a]quaternary pump
[b]binary pump

Vanquish UHPLC platform parts are listed below. Part numbers are provided in the link below.

- Vanquish autosampler loop sizes and gradient delay volumes
  - 250 µL loop: 450 µL
  - 100 µL loop: 290 µL
  - 25 µL loop: 135 µL
  - 10 µL loop: 93 µL
- Vanquish active column preheater (total volume including tubing):
  - 0.1 mm x 380 mm: 3 µL
- Vanquish Core/Flex optional pump kits:

  Static mixers for use with 50 µL capillary mixer:
  - Static mixer volume: 150 µL (for total volume of mixing system: 200 µL)
  - Static mixer volume: 350 µL (for total volume of mixing system: 400 µL)
  - Static mixer volume: 750 µL (for total volume of mixing system: 800 µL)
  - Static mixer volume: 1,500 µL (for total volume of mixing system: 1,550 µL)
- Vanquish Horizon optional pump kits:

  Mixer kits:
  - Kit volume: 200 µL (Static: 150 µL, Capillary: 50 µL)
  - Kit volume: 400 µL (Static: 350 µL, Capillary: 50 µL)

Changing the loop size and mixer should be enough to meet system suitability requirements, but if needed, some capillaries are available. There are two types of capillaries offered:

- Thermo Scientific™ Viper™ Fingertight Fitting System:

  Stainless steel (<1,300 bar); MP35N (<1,500 bar):
  - Internal diameters: 0.100 mm to 0.180 mm
  - Lengths: 65 mm to 950 mm

Depending on the needed gradient delay volume adjustment, the Viper capillary contribution can be calculated:

Volume of cylinder (V) = $\pi r^2 h$

Tubing volume (1 mm^3) = 1 µL

- For tubing 0.065 mm × 150 mm:

  Volume = 3.1415 • 0.03252 • 150 = 0.5 mm^3 = 0.5 µL
- For tubing 0.18 mm × 650 mm:

  Volume = 3.1415 • 0.092 • 650 = 16.5 mm^3 = 16.5 µL

Example: Adjusting the Vanquish Flex system to match the Waters Alliance system

Waters Alliance gradient delay volume: 1,100 µL

Vanquish Flex recommendations:

- Determine pump and injection volume configuration:
  - For this example, a quaternary pump and a 100 µL injection loop is used.
  - This results in a default gradient delay volume of 974 µL.
- Determine remaining gradient delay volume needed:
  - 1100 – 974 = 127 µL
- Assess chromatography and determine if additional adjustment is needed.

*The current P/N of these parts can be found at: https://www.thermofisher.com/us/en/home/industrial/chromatography/liquid-chromatography-lc/hplc-uhplc-related-products/fittings/selection.html?category=tubing

Instrument-to-instrument qualification guidance

Instrument-to-instrument transfers, and the associated modifications to the system, often do not require full requalification. However, please note that all local and corporate SOPs should be followed when designing experiments to qualify instruments!

Scenario 1

Method validated, new instrument to be qualified, no retention time requirements in method and/or quality agreement or SOPs do not require official qualification study.

- Perform system suitability on an already approved instrument and the new instrument using the same analyst, solutions, column.
- Verify chromatography is consistent prior to proceeding with sample analyses (same relative retention time, no unexpected peaks, etc.).
- Verify sample results are consistent between systems.

Scenario 2

Method validated, new instrument to be qualified, retention time requirements in method and/or quality agreement or SOPs require official qualification study.

- Draft supplemental validation protocol to perform system suitability and sample analysis on an already approved instrument and the new instrument using the same analyst, solutions, column.
- Apply similar criteria from intermediate precision or robustness experiments from original validation.

Example of Scenario 2: System comparison study

The method to be performed in this study was validated using an HPLC system from an outside vendor. A Vanquish system is designed to be equivalent/superior to the outside vendor system. The system comparison study will evaluate the suitability of a Vanquish Flex to be used in execution of the method.

Experimental procedure:

1. Analyst-1 will prepare solutions, standards, and samples as indicated in the method. These solutions will be used for Steps 2 and 3.
2. Analyst-1 will perform an analysis on the outside vendor system.
3. Analyst-1 will perform an analysis on a Vanquish Flex system using the same column used in Step 2.

Acceptance criteria:

1. The system suitability requirements of the method must be met for each analysis performed during the study for the study to be considered valid.
2. The elution order of known and unknown peaks must be consistent between systems.
3. The number of reportable impurity peaks detected in sample preparations must be consistent between systems.
4. The assay results obtained on the Vanquish Flex system must be within 1.0% absolute difference of the assay results obtained on the outside vendor system.
5. The impurity results obtained on the Vanquish Flex system must meet the criteria in Table 8.

Table 8. Acceptance criteria

Average % impurity result	Acceptance criterion
> 0.25%	Absolute difference from impurity results from outside vendor system ≤ 0.10%
≤ 0.25%	Absolute difference from impurity results from outside vendor system ≤ 0.05%

Scenario 3

Method to be developed on multiple systems

- As part of robustness testing, perform system suitability and sample analysis on different instruments using the same analyst, solutions, column.
- Apply similar criteria from other robustness experiments.

Scenario 4

Method to be validated on multiple systems

- As part of intermediate precision or robustness testing, perform system suitability and sample analysis on different instruments using the same analyst, solutions, column.
- Apply similar criteria from other intermediate precision or robustness experiments.

Transferring methods to the Vanquish platform improves overall analytical performance

Now that guiding principles and best practices for liquid chromatography method transfers have been examined, practical examples for different assays that demonstrate performance improvement in the laboratory will be discussed.

Decreasing system suitability failures and improving resolution

Table 9 shows the system suitability requirements and how the Vanquish Flex performance compares to the original Waters ACQUITY H-Class system. It should be noted that the Vanquish Flex system improves on tailing, which leads to fewer system suitability failures and longer column lifetime.

Improving sensitivity and reducing system suitability failure

Table 10 shows the system suitability requirements and how the performance of the Vanquish Flex system compares to the two other systems. As indicated, the Vanquish Flex system shows increased sensitivity, but more importantly, significantly reduces system suitability failures when compared to the Agilent 1260 Infinity II system, which failed system suitability repeatedly.

Table 9. System suitability summary for Vanquish Flex system and Waters AQUITY H-Class system

Parameter	Criteria	Vanquish Flex Quaternary	Waters ACQUITY H-Class
No significant interference at RT of active and impurities in blank injection	NMT 0.1% of active area in 1st standard injection	No interference	No interference
USP S/N of sensitivity	NLT 10	31	35
Theoretical plates (n=5)	NLT 10,000	52,444	55,713
Tailing factor (n=5)	NMT 2.5	**2.1**	**2.4**
%RSD of active peak area (n=5)	NMT 2.0%	**0.0**	**0.2**
%RSD of active peak area (n=all)	NMT 2.0%	**0.1**	**0.4**
%RSD of active RT (n=5)	NMT 2.0%	**0.0**	**0.1**
%RSD of active RT (n=all)	NMT 2.0%	**0.0**	**0.1**
Check standard (% Recovery)	98.0–102.0%	99.7	100.4
Resolution between impurity A and active peak	NLT 1.0	**1.2**	**1.1**

Table 10. System suitability summary for all three systems

Parameter	Criteria	Vanquish Flex	Waters ACQUITY H-Class	Agilent 1290 Infinity II
USP S/N of sensitivity	NLT 10	**17**	**15**	**12**
Tailing factor (n=5)	NMT 2.0	1.0	1.0	1.1
%RSD of active peak area (n=5)	NMT 2.0%	0.0	0.2	0.1
%RSD of active peak area (n=all)	NMT 2.0%	0.1	0.2	0.3
%RSD of active RT (n=all)	NMT 2.0%	0.0	0.0	0.0
Check standard (% Recovery)	98.0–102.0 %	100.1	100.0	100.0
Resolution between impurity A and impurity B	NLT 1.0	2.1	2.2	2.1

Conclusion

Method transfer should not be cumbersome. With good knowledge of regulatory requirements, an understanding of how the target liquid chromatography system compares to the origin system, and with documented and characterized system modifications, method transfer should no longer be a bottleneck to modernization. More importantly, modernizing methods on the Vanquish platform can lead to significant improvements to an analytical method, such as better resolution, improved sensitivity, and reduced system suitability failures.

Reference

1. Thermo Scientific Case Study 000565: Overcoming the challenges of liquid chromatography method transfer: A CDMO perspective.

Learn more at **thermofisher.com/HPLC**

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