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Analytical Method Transfer to Modern UHPLC Instruments

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ABSTRACT

Purpose: To develop a systematic approach for method transfer and modernization onto the Thermo Scientific™ Vanquish™ UHPLC platform as a strategy to address method life-cycle management, a regulatory requirement of the pharmaceutical industry.

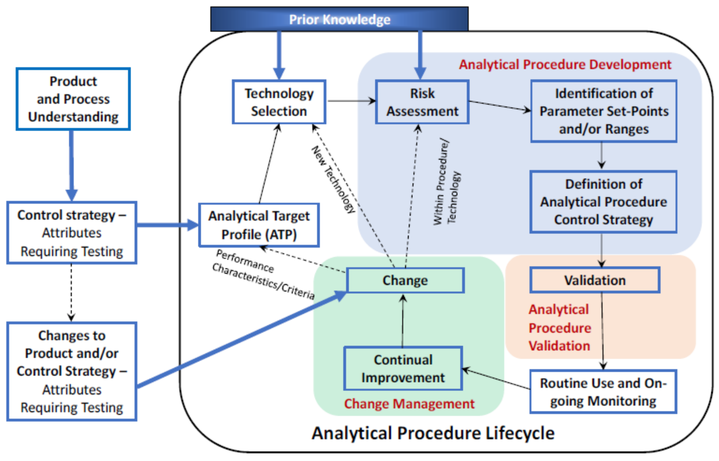
Methods: Gradient delay volumes of the originator system and the target liquid chromatography system were assessed. This was done by knowing the exact configuration of the originator system and comparing it to the Vanquish™ UHPLC system configuration. In cases where the gradient delay volume of the originator system was not known, it was evaluated experimentally. Chromatography between the originator system and the Vanquish™ was then reviewed to ensure system suitability requirements (SST) of the method were met.

Results: Approximately 200 HPLC and UHPLC methods for both chemical medicines and biotherapeutics ranging from early development phases to commercial products were transferred onto the Vanquish™ UHPLC platform within the analytical development and quality control laboratories of the Thermo Fisher Scientific Pharma Service Group (PSG, also known as Patheon). The new systems offer improved sensitivity, flexibility and reduced system suitability failures through reduced baseline fluctuation cause by both pump and injector.

INTRODUCTION

The ICH Q14 guidance on Analytical Procedure Development[1] (currently under review with the industry) provides general recommendations for analytical procedure development and lifecycle management. In short, the goal of development is to obtain analytical procedure **fit for its intended purpose** and two approaches: **minimal** and **enhanced** to analytical method development should be considered. Although, the minimal approach is acceptable in most cases, the enhanced approach is ideal to support development and lifecycle management of analytical procedures by offering a systematic way of developing and redefining knowledge of analytical procedures. The analytical product lifecycle comprises several elements like the analytical procedure development, validation and change management which are interrelated as shown in figure 1.

Figure 1. Analytical Procedure Lifecycle (taken from ICH Q14 section 2.3)



Part of change management is continual improvement of the analytical procedure, which can be achieved by modernizing instrumentations and transferring analytical methods to the latest technologies leading to improved specificity, enhanced precisions, accuracy and overall lab efficiency gains.

Analytical method transfer is the documented process that qualifies a receiving laboratory to execute testing with an analytical method that originated in another laboratory. The method transfer process can also be applied to qualify new equipment by comparing chromatographic results obtained between new and originating instruments. This can be a cumbersome and time-consuming endeavor, but with a mechanistic understanding of the instruments and the test methods being qualified, suitability and optimization of the instrument and method can be achieved.

When transferring methods, it is important to be aware of what modifications are allowable as per regulatory requirements. For compendial methods, recent updates to USP Chapter <621> state that *"adjustments to the specified chromatographic system may be necessary in order to meet system suitability requirements. Adjustments are permitted only when suitable standards (including Reference Standards) are available for all compounds used in the suitability test, and the adjustments or column change yields a chromatogram that meets all the system suitability requirements specified in the official procedure…"*[2]. If adjustments are necessary, 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 are allowed. For non-compendial methods, modifications with the method operable design range (MODR) or robustness should be allowed. Robustness is best determined by analytical quality by design (AQbD) experimentation during method development. Modern software packages like ChromSword™ Chromeleon Connect software and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) can be used to facilitate the robustness process.

There are two approaches to compensate for gradient volume differences 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.

METHODS

Determining Originator Instrument Gradient Delay Volumes (GDV)

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 volumes (GDVs) can be calculated if volumes of each pump and autosampler components are known. Alternatively, if the configuration of the originator system is not known, the gradient delay volume can be measure experimentally as follows:

1. Run a gradient from 0% solvent B to 100% solvent B (inject solvent A or 0 µL) as indicated in Table 1.
   - 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

   Note: Adjusting the isocratic hold times at the beginning and end of the gradient to capture the system delay might be needed.

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

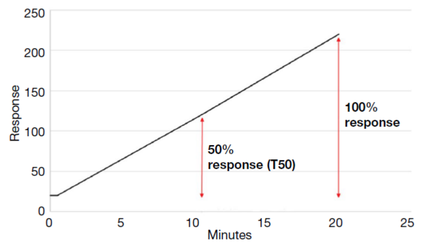
T50 = Time of 50% response

TG = Time of Gradient (exclude hold times)

Table 1. Experimental Delay Volume Gradient

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

Figure 3. Experimental Delay Volume Gradient Response



For example, using value from figure 3, we obtain:

0.5 mL/min × (10.5 min – (0.5 × 20 min))
0.5 mL/min × 0.5 min
= 0.25 mL or 250 µL

Once the Gradient Delay Volume of the originator instrument is known, a comparison can be made to the new instrument as in the example below.

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

Waters Alliance gradient delay volume: 1,100 µL

Vanquish Flex recommendations:

- Determine pump and injection volume configuration (refer to Figure 4):
  - 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 µL – 974 µL = 127 µL
- Assess chromatography and determine if additional adjustment is needed.

Figure 4. 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		

Further information on different configurations as well as standard gradient delay volumes of various liquid chromatography systems can be found in reference 3.

RESULTS

This systematic approach has led to the transfer of approximately 200 HPLC and UHPLC methods without needing to perform significant method revalidation and qualification. The novel instruments are not only more robust, flexible and easy to maintain, they provide significant benefits in terms of analytical performance improving day to day lab operations.

Improved Instrument Performance Reduces System Suitability Failures

Failing to meet system suitability requirements (SST) is a significant challenge in cGMP laboratories. This can lead to out of specifications results and deviations as the cause of the system suitability failures needs to be investigated. The process can be lengthy and leads to instrument being unuseable. They are standard practices that can be put in placed to avoid such events, for example having proper sample preparation techniques, using suitable UHPLC Grade solvent and clean glassware. However, when it comes to instrumentations, its more difficult to take actions to proactively prevent system suitability failures, especially with older instrumentations. For example, as seen in table 2, a UHPLC of another brand was very close to the tailing factor requirements of the method, which lead to frequent system suitability failures. Fortunately, transferring the method to a Vanquish Flex UHPLC system reduces the tailing factor and thus meeting system suitability criteria more consistently. The tailing reduction also improved the column lifetime as the system provided a larger operating range before the column performance led to system suitability failure due to tailing.

Figure 2. Vanquish Flex UHPLC

Table 2. Reduce tailing improves system suitability

Parameter	Criteria	Vanquish Flex	UHPLC A
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	52444	55713
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**

Transferring methods to the Vanquish Flex system also proved beneficial in improving the signal-to-noise ratio (method sensitivity). As seen in table 3, the s/n ratio for the method ran on a HPLC was very close to the allowed limit of the method resulting in several system suitability failures during operation on older HPLC systems. Fortunately, moving the method to the Vanquish Flex significantly improved sensitivity and thus reduced system suitability failures.

Table 3. Improve Signal to Noise Ratio improves system suitability

Parameter	Criteria	Vanquish Flex	HPLC
USP s/n of Sensitivity	**NLT 10**	**17**	**12**
Tailing Factor (n=5)	NMT 2.0	1.0	1.1
%RSD of Active peak area (n=5)	NMT 2.0%	0.0	0.1
%RSD of Active peak area (n=all)	NMT 2.0%	0.1	0.3
%RSD of Active RT (n=all)	NMT 2.0%	0.0	0.0
Check Standard (% Recovery)	98.0-102.0 %	100.1	100.0
Resolution Between Impurity A and Active Peak	NLT 1.0	2.1	2.1

Intermediate Precision: Vanquish Flex (A) to HPLC (B)

The Vanquish flex system is designed for UHPLC performance but is also suitable for running legacy HPLC methods. Figure 4 shows the results of an intermediate precision study to assess the performance of a Vanquish Flex UHPLC to meet the requirements of a HPLC method. The method was run by different analysts, samples, solutions and provided the same chromatographic results on the two systems. Moreover, the Vanquish Flex showed improvement in %RSD and s/n ratio of LOQ (table 4.).

Figure 4. Intermediate Precision: Vanquish Flex (A) to HPLC (B)

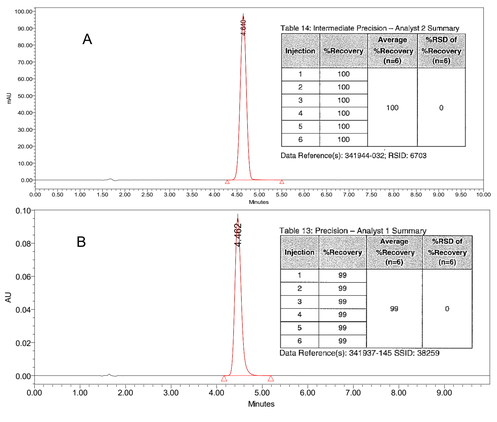


Table 4. Intermediate Precision Vanquish Flex to UHPLC

Parameter	Vanquish™ Flex	HPLC
USP s/n of LOQ	**40**	**32**
%RSD of Active peak area (n=5)	0.1	0.1
%RSD of Active peak area (n=all)	**0.1**	**0.5**
USP Tailing	1.0	1.2

CONCLUSIONS

- A systematic approach for transferring liquid chromatography analytical methods was put in place to transfer approximately 200 HPLC and UHPLC methods with little or limited re-validation and re-qualification.
- A mechanistic understanding of the instruments and test methods being qualified as well as a good understanding of regulatory requirements reduces the challenges associated with method transfer and modernization.
- Modernizing analytical methods has significantly improved daily laboratory operations by improving overall analytical performance, flexibility, and robustness resulting in reduced system suitability failures.

REFERENCES

1.ICH Q14 – Analytical Procedure Development, Draft Version, Endorsed on 24 March 2022, ICH 2022 https://database.ich.org/sites/default/files/ICH_Q14_Document_Step2_Guideline_2022_0324.pdf
2. USP <621> "Doc_ID: 1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_1_en-US ".
3. Thermo Scientific Case Study 000566 Method Transfer onto the Vanquish UHPLC Platform: A CDMO Perspective.

TRADEMARKS/LICENSING

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