Liquid chromatography

Overcoming the challenges of liquid chromatography method transfer: A CDMO perspective

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Introduction

Analytical method transfer and method modernization can be a barrier to upgrading to the latest technologies. Revalidating an existing method while continuing to meet regulatory guidance can be a challenging and time-consuming endeavor. However, method modernization is often less difficult than the common perception, and good practices can be put in place to streamline and facilitate the process. This case study will address some of the concerns related to method transfer and provide guidance from a leading contract development and manufacturing organization (CDMO) authority. A method transfer guide onto the Thermo Scientific™ Vanquish™ UHPLC platform (CS000566)[1] based on these guiding principles complements this document.

Thermo 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. Analytical method transfer is an integral part of PSG drug development support, and by combining current regulatory guidance, industry best practices, and an understanding of the liquid chromatography platforms, successful method transfers are accomplished.

Quality should be built into the design of a method

A commonly adopted definition of method transfer comes from Rozet,[2] who defines it as "the process of transferring a validated analytical method from a sending laboratory to a receiving laboratory, after demonstrating experimentally that it also masters the method." In other words, the transferred method must meet or improve upon the requirements of the initial method, and the method must remain fit for its intended purpose. By understanding the differences between the origin and target systems, the transfer of the method should not present a significant challenge for the analyst. It should be noted that instruments from different vendors and configuration differences from one laboratory to another will often exhibit inherent differences, such as gradient delay volume or mixing behavior. Therefore, transferring a method between two different LC systems could lead to chromatographic differences, which need to be addressed, or at least, documented. A thorough understanding of regulatory guidance and the adoption of an Analytical Quality by Design (AQbD) approach can go a long way in developing robust methods from inception.

ICH Guidance on Quality by Design (ICH Q8(R2) 2. PHARMACEUTICAL DEVELOPMENT) states that **quality cannot be tested into products, i.e., quality should be built in by design.** Changes in formulation and manufacturing processes during development and lifecycle management should be looked upon as opportunities to gain additional knowledge and further support establishment of a method design space. Similarly, inclusion of relevant knowledge gained from experiments giving unexpected results can also be useful. The design space is proposed by the applicant and is subject to regulatory assessment and approval. It should be noted that working within the method design space is not considered as a change. There is currently no chapter for analytical method development from the ICH. However, guidance documents are planned and will adapt similar expectation for Analytical Development (Q14-pending). **The FDA already accepts and expects analytical QbD for all new drug applications (NDAs). Analytical method lifecycle management is expected** alongside formulation and manufacturing processes for product lifecycle management.

ICH does however provide some guidance on established conditions (EC) of methods (ICH Q12 3.2.3.2 Identification of ECs for Analytical Procedures). Similar to the principles described for manufacturing processes, ECs related to analytical procedures should include elements that assure performance of the procedure. **The extent of ECs and their reporting categories could vary based on the degree of the understanding of the relationship between method parameters and method performance, the method complexity, and control strategy.** A justification to support the identification of ECs and corresponding reporting categories for changes to ECs based on risk management should be provided. Different approaches can be used to identify ECs for analytical procedures, for example as analytical technology and development approaches advance; these approaches include, but are not limited to, the following:

- When limited development studies have been conducted, this may result in a narrow operating window to ensure method performance. In such cases, ECs may be more extensive with fixed and/or tight conditions.
- **Enhanced understanding can lead to a wider operating window** that ensures method performance, where ECs can be reduced and focused on method performance (e.g., method parameters' acceptable ranges rather than set points, performance criteria).

ECs or operating parameters for analytical methods can be varied within the design space (robustness) of the method without the modifications being considered changes. Better mechanistic understanding of the method leads to better operating parameters and better method performance. ECs can be instrument parameters, such as flow rate, column temperature, gradients, etc., or mobile phase solution concentrations and pH.

Robustness is best determined by analytical quality by design (AQbD) experimentation during method development. For older methods, with a single parameter varied per robustness analysis, this may be tedious as it typically allows only a single modification at a time. It is recommended that high volume methods should adopt an AQbD approach to re-define the robustness range. There are available software systems compatible within existing CDS environments, such as the connectivity between ChromSword Chromeleon Connect software and Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), to facilitate the process.

Practical example of method modification within the design space

A recent issue arose in the PSG Analytical Development Group where new batches of a particular column showed greater retention of a single known impurity (peak-A) than previous batches of these columns (from the same manufacturer, stationary phase, particle size, and column dimension). These retention time changes caused loss of resolution between the known impurity peak-A and another peak of interest (peak-B) (Figure 1A).

Fortunately, the composition of mobile phase A was evaluated during robustness analysis to be suitable for up to a +10% relative increase in acetonitrile content. Mobile phase A preparation was therefore adjusted from 33% acetonitrile to 34% (+3% relative increase equivalent to +1% absolute increase) to obtain the desired resolution between the 2 known impurity peaks (Figure 1B).

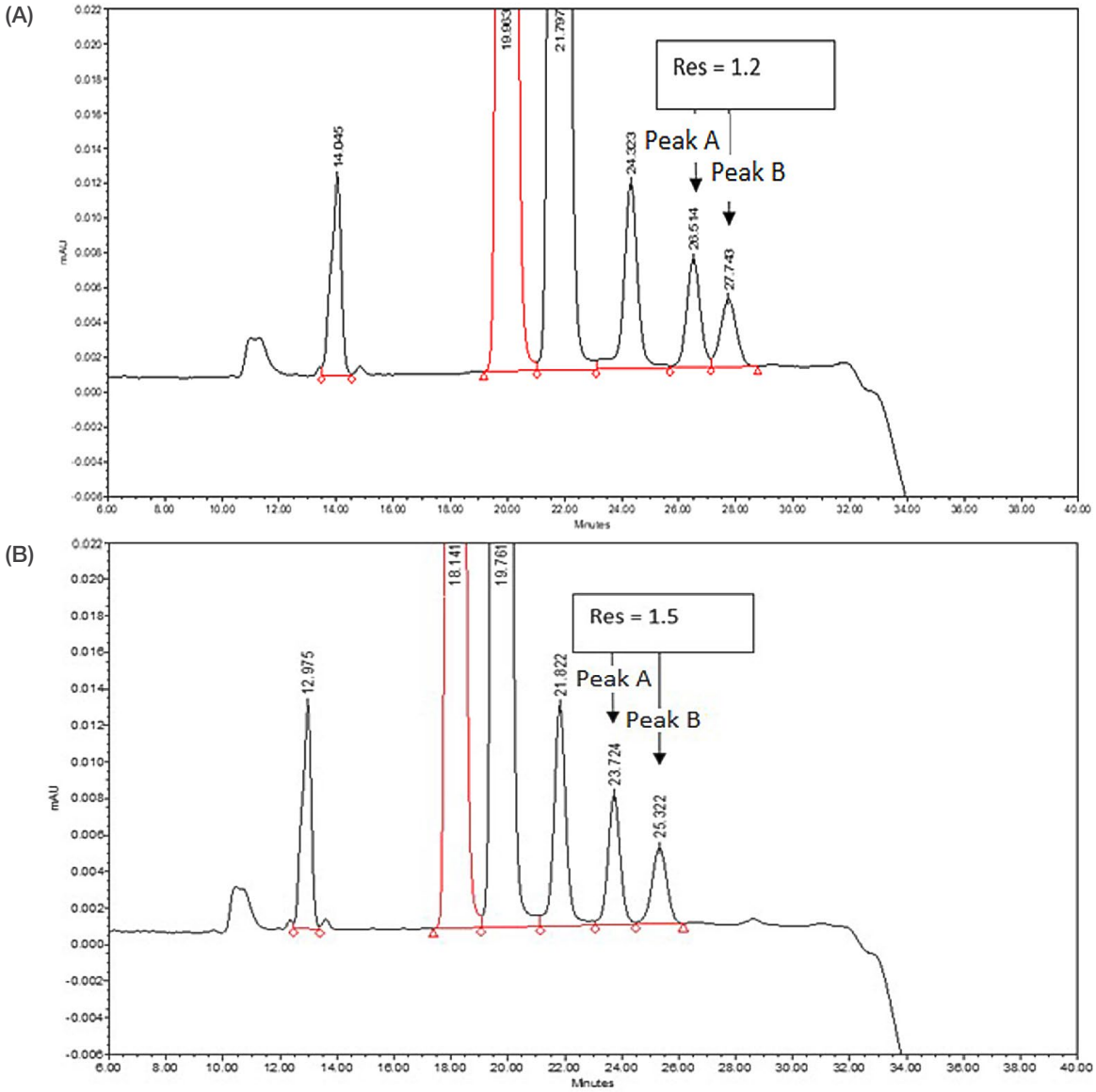

Figure 1. (A) Loss of resolution between peaks A and B; (B) Resolution improvement between peaks A and B by increasing ACN concentration in solvent A by 1%, allowable per robustness analysis

Without suitable method robustness, method evaluation, and a mechanistic understanding of the method established conditions, this issue could have led to potential deviations and the need to repeat method validation experiments to qualify another column or mobile phase conditions.

Working from compendial methods

When the compounds of interest have an existing monograph, an analyst can rely on guidance from an entity such as USP or EP. Recent updates to USP Chapter <621>[3] 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…"*

To verify the suitability of the method under the new conditions, an analyst must assess the relevant analytical performance characteristics potentially affected by the change. Multiple adjustments can have a cumulative effect on the performance of the system and are to be considered carefully before implementation. Some modifications, like adjustments to the

composition of the mobile phase in gradient elution that may cause changes in selectivity, are not recommended for gradient methods. 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.

Table 1 summarizes some allowed changes for both isocratic and gradient methods. Any change of the method parameters beyond the range described below usually requires the full re-validation.

Although changing in the mobile phase is not recommended for gradient methods, it is not explicitly prohibited. Based on the USP guidance, the concentration of minor components of the mobile phase (specified as ≤50%) can be modified. These components can be adjusted by ±30% relative. However, the change in any component cannot exceed ±10% absolute (i.e., in relation to the total mobile phase). For a ternary mixture, adjustment can only be made to a single minor component. Examples of adjustments for binary and ternary mixtures follow.

Binary mixtures

Specified ratio of 50:50: 30% of 50 is 15% absolute, which exceeds the maximum permitted change of ±10% absolute in either component. Therefore, the mobile phase ratio may be adjusted only within the range of 40:60–60:40.

Specified ratio of 2:98: 30% of 2 is 0.6% absolute. Therefore, the maximum allowed adjustment is within the range of 1.4:98.6–2.6:97.4.

Ternary mixtures

Specified ratio of 60:35:5: For the second component, 30% of 35 is 10.5% absolute, which exceeds the maximum permitted change of ±10% absolute in any component. Therefore, the second component may be adjusted only within the range of 25%–45% absolute.

For the third component, 30% of 5 is 1.5% absolute, which meets the allowed requirement for a single component. In all cases, a sufficient quantity of the first component is used to give a total of 100%. Therefore, mixture ranges of 50:45:5–70:25:5 or 58.5:35:6.5–61.5:35:3.5 would meet the requirement.

Table 1. Summary of USP adjustment guidelines

		OK for	
Component	Allowed range	Isocratic	Gradient
Mobile phase minor component (≤50%)	±30% relative; Cannot exceed ±10% absolute change; Cannot be reduced to zero	Yes	NR*
Mobile phase pH	±0.2 pH units	Yes	Yes
Buffer concentration	±10%	Yes	Yes
Column temperature	±10 °C	Yes	Yes
Injection volume	Can be adjusted as needed as long it is consistent with linearity, precision, and detection requirements	Yes	Yes
Detector wavelength	NA	No	No
Flow rate	±50% (at given ID)	Yes	No
Column inner diameter	Can be adjusted as long as linear velocity is maintained	Yes	No
Column length and particle size	Column length (L) to particle size diameter (dp) can be adjusted between -25% to +50%	Yes	No
Stationary phase	No change of the identity of the substituent permitted	No	No
Guards	Same stationary phase as column; guard ID ≤ column ID; Guard length ≤ 15% column length	Yes	Yes

*NR = Not recommended, but not explicitly prohibited

Conclusion

As discussed, 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. This topic, as well as a real-life example, guidance, and easy to implement practices, is covered in Case Study 000566.

References

1. Thermo Scientific Case Study 000566 Method Transfer onto the Vanquish UHPLC Platform: A CDMO Perspective.
2. Rozet, et al., *J. Chrom. B,* **2009,** *877* 2214–2223.
3. USP <621> "Doc_ID: 1_GUID-6C3DF8B8-D12E-4253-A0E7-6855670CDB7B_1_en-US ".

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