TECHNICAL NOTE 73371

Physical adjustment of gradient delay volume as a tool for successful transfer of HPLC methods

Authors: Tibor Muellner and Holger Franz, Thermo Fisher Scientific, Germering, Germany

Keywords: method transfer, gradient delay volume, adjustable GDV, metering device, idle volume, method transfer kit, regulatory compliance

Introduction

The transfer of chromatographic methods from an older HPLC instrument to a newer generation instrument or the transfer of methods between instruments of different vendors are challenging tasks for many HPLC practitioners. Differences in the hardware designs can affect chromatographic results, such as the thermostatting mode of the column compartment, the eluent pre-heating, and for gradient methods most importantly the instrument volumes as defined by the utilized pump technologies, autosampler designs and system plumbing[1].

The tools and possibilities for a user to mitigate the impact of instrument differences on validated chromatography methods are usually limited by regulatory bodies. For example, the United States Pharmacopeia (USP) Chapter <621> lists the permitted changes to a method without the need for revalidation and is often used as a reference for the degrees of freedom during HPLC method transfer[2]. Further changes which encompass specific system settings (e.g. forced air vs. still air thermostatting of a column oven) or mechanical changes to an instrument (e.g. changing a passive with an active column preheater) are usually considered acceptable as long as the instrument is qualified using a given configuration.

For gradient HPLC methods the instrument gradient delay volume (GDV) plays a crucial role for a successful method transfer. The GDV is defined as the volume from the point of mixing of the eluents to the column head[1]. Changes to the GDV or the length of an isocratic hold are explicitly permitted according to USP chapter <621>. Therefore, these changes are popular tools for successful method transfer. Changes to the isocratic hold are not universally applicable though, as they are limited to applications where an isocratic hold is prescribed[2]. Software-based compensation of differences in GDV, usually by a time-shift of the injection event or an alteration of the gradient profile can raise concerns of regulatory authorities.

Gradient delay volume adjustment strategies

Manual changes to the gradient delay volume

Common strategies for changing the GDV of a system consist of adding large volume fluidic components into the flow path, i.e. placing mixers or large volume capillaries between the pump and the autosampler. While these changes usually help mimick a source instrument, the downsides are that mixers and capillaries have fixed volumes and thus do not allow for an exact setting of the GDV. Particularly in regulated environments, these hardware changes mandate a (re)qualification of the altered instrument and thus frequent changes to the system hardware is impractical. Consequently, this means that often a system configuration is locked to a fixed hardware configuration.

Switching of flow paths with different gradient delay volumes

Several commercial products try to circumvent this limitation by providing two separate flow paths; one providing a low GDV for short gradient responses required for applications using narrow-bore HPLC columns, and one large GDV flow path dedicated to providing compatibility with legacy methods/ HPLC instruments. Thus, with these products, the user can switch between full performance and legacy compatibility. Drawbacks of this approach are

1. striving for minimum GDV may lead to lower mixing performance,
2. both flow paths are static and cannot be further adjusted,
3. the instrument is optimized to transfer legacy methods from a single source system type only.

Freely tunable gradient delay volume

Thermo Scientific™ Vanquish™ Core HPLC systems use a metering device in the autosampler to aspirate the sample before the injection. The metering device is part of the flow path and contributes to the GDV of the HPLC system. The flushed through volume of the metering device can, however, be altered by the movement of a piston, thus changing the overall GDV of the HPLC system. This flushed through volume is called idle volume. The idle volume can be decreased by a software command to a low volume to mimic a system with a small GDV (Figure 1, left) or increased to a high volume to simulate a system with a higher GDV. The set idle volume and the injection volume are independent of each other, i.e. any combination of both is possible. The setting of the idle volume is tracked by the Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software audit trail (version 7.2.10 MUa and higher) and therefore fully auditable. This functionality is also available with Thermo Scientific™ Standard Instrument Integration (SII) for Thermo Scientific™ Xcalibur™ software (version 1.6).

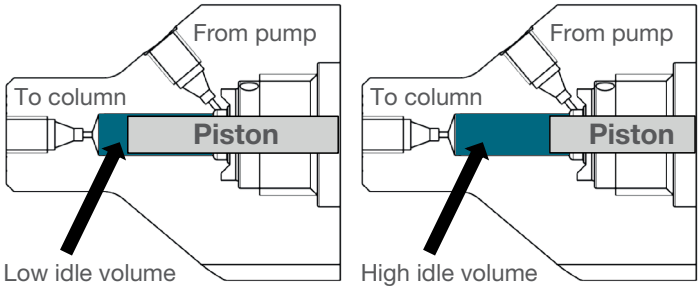

Figure 1. Effect of the metering device piston position on the gradient delay volume. The geometry is designed to ensure that, independent of the piston position, the entire idle volume is flushed, i.e. the geometry prevents stagnant zones from occurring, throughout all piston positions (idle volumes) and flow rates.

As the Vanquish Core system has a lower GDV than most routine HPLC systems, which typically have a GDV in the range of 1.1 to 1.4 mL (for low-pressure mixing pumps), the idle volume setting of up to 230 µL is usually sufficient to compensate GDV differences. A typical example is shown in Figure 2, where the idle volume setting was utilized to transfer a method for impurities in chlorhexidine from an Agilent 1260 Infinity LC system to a Vanquish Core HPLC system. By adaptation of the idle volume, the match of peak retention times could be greatly enhanced. For details of this method transfer example, refer to Application Note 73309: Straightforward transfer of an EP method for chlorhexidine impurity analysis from an Agilent 1260 Infinity LC system to a Vanquish Core HPLC system[3].

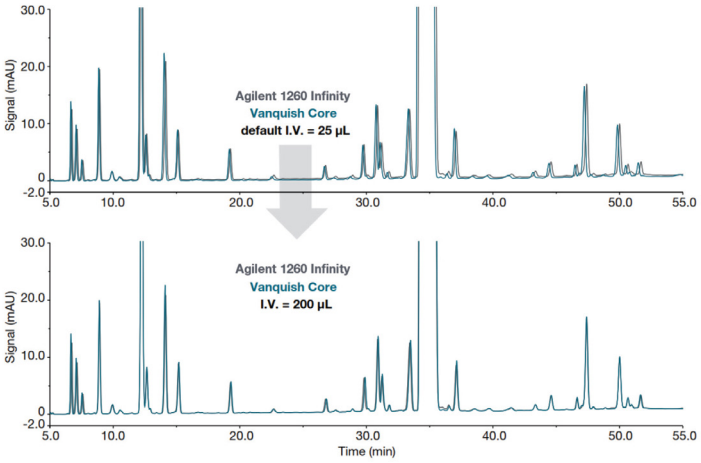

Figure 2. Example transfer from an Agilent 1260 Infinity Series HPLC system to a Vanquish Core HPLC system of a method determining impurities in chlorhexidine. Excellent overlap of the results could be achieved by increasing the system GDV by 175 µL. Reproduced from Application Note 73309: Straightforward transfer of an EP method for chlorhexidine impurity analysis from an Agilent 1260 Infinity LC system to a Vanquish Core HPLC system[3].

To further extend the GDV flexibility, a Method Transfer Kit (P/N 6036.2100) is available for Vanquish Core HPLC systems. It consists of a 6-port 2-position switching valve and a 200 µL loop which can be inserted into the flow path. The fluidic setup is depicted in Figure 3. Switching of the valve allows to increase the gradient delay volume by additional 200 µL. As this additional volume is placed before the point of injection, the system dispersion is not impacted.

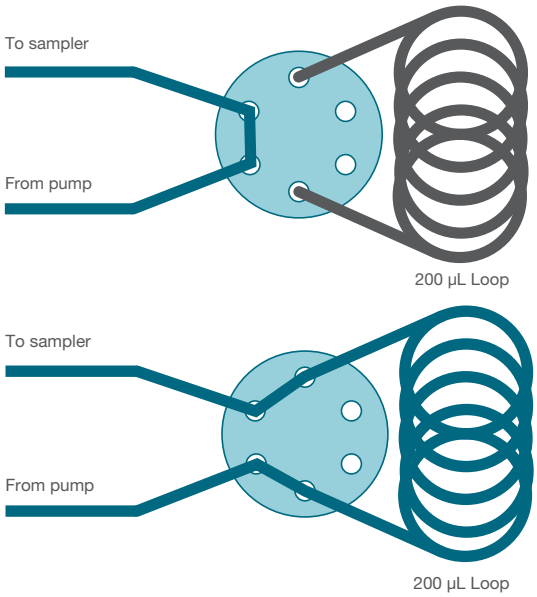

Figure 3. Switchable additional gradient delay volume. In bypass position, the flow from the pump is directly connected to the sampler, analogue to an instrument without installed method transfer kit (top). When the valve is switched, the loop is part of the flow path, adding the loop volume to the overall gradient delay volume of the system (bottom).

The GDV adjustment through the idle volume of the metering device of the Vanquish Core system is now combinable with the switching of the loop into the flow path. A combination of the two functions allows a 1 µL step tuning of the system GDV over a range of 430 µL decreasing it by 25 µL and increasing the system GDV by 405 µL compared to the default configuration. This is achieved by adjusting the idle volume of the metering device, the 200 µL loop, or a combination of both. This concept is also visualized in Figure 4.

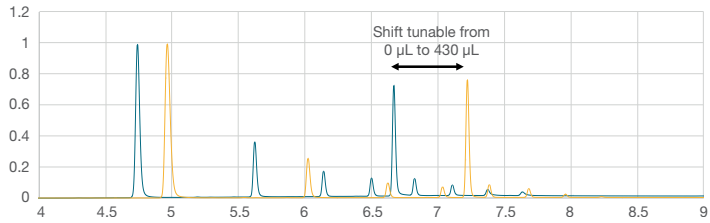

Figure 4. Impact of different GDV on a gradient separation using a Vanquish Core HPLC system. The lowest GDV can be achieved by an idle volume setting of 0 µL while switching the method transfer loop out of the flow path. The highest GDV can be implemented by an idle volume setting of 230 µL and simultaneously switching the loop into the flow path, the adjustment of the GDV is possible by 1 µL steps in a range of 430 µL.

Ease-of-use and software implementation

Whenever a Vanquish Method Transfer Kit (P/N 6038.2100) is installed, the system GDV can be influenced by using the loop, metering device, or both. To install the kit, it must be configured in the Chromeleon CDS (version 7.2.10 MUa or later) instrument configuration, as shown in Figure 5.

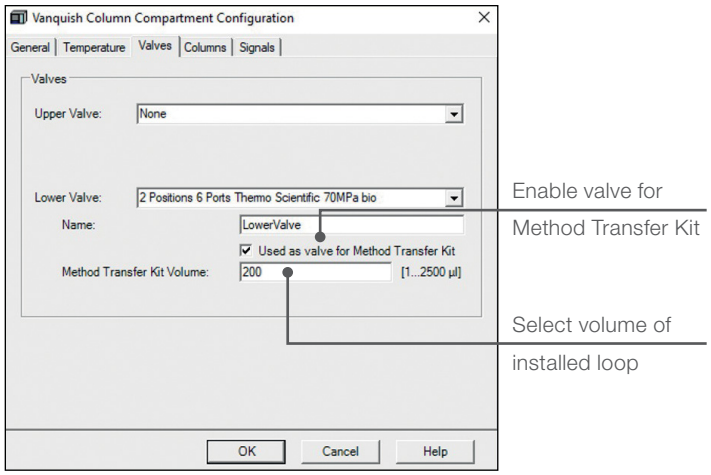

Figure 5. Instrument configuration screen of Chromeleon 7.3 CDS allowing the configuration of the method transfer kit and defining the loop volume thereof. 200 µL is the volume of the loop provided with the method transfer kit, other volumes for customized loops can also be entered.

The volume of the loop shipped with the method transfer kit is 200 µL. In the Chromeleon software instrument configuration, it is also possible to define a different volume, for instance for a custom loop.

The Chromeleon Instrument Method Editor provides easy access to adjust the GDV as a regular method parameter, like for instance the sample temperature of the column compartment. This allows setting of the GDV within an instrument method and therefore individually for a sequence or even a specific sample. Figure 6 shows screen captures of the Chromeleon Instrument Method Editor (top) and Instrument Method Wizard (bottom) to illustrate this. The setting of the valve position and idle volume setting is fully tracked in Chromeleon CDS audit trails and can therefore be audited at any time.

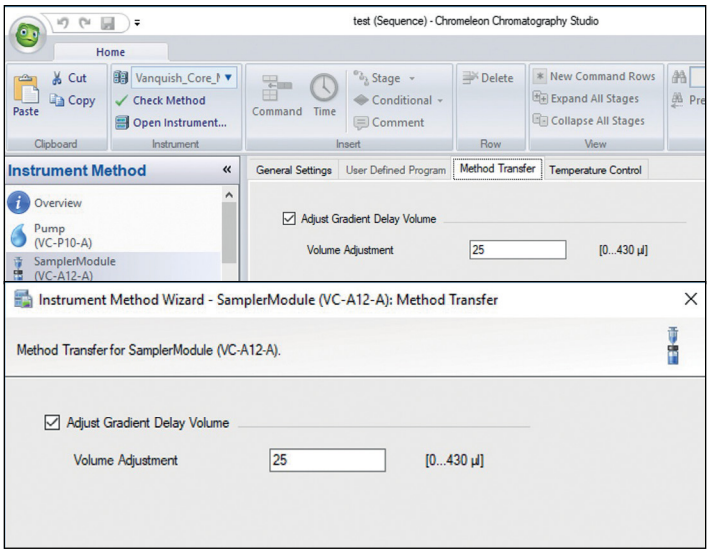

Figure 6. Chromeleon Instrument Method Editor (top) and Instrument Method Wizard (bottom), allowing to adjust the gradient delay volume of the system during method editing and method creation.

Limitations to using the gradient volume for method transfer

For a given gradient HPLC method, a change to the GDV or the length of the isocratic hold at the beginning of a method, can influence retention times across a chromatogram in an inconsistent way. Peaks eluting during the isocratic hold or close to it undergo a full or partial isocratic elution mechanism. Their retention times are usually not or minorly affected by GDV changes. Peaks eluted fully by the impact of the gradient typically show a deviation in accordance with the GDV difference. Both effects in a chromatogram can influence the chromatographic resolution.

To illustrate this effect, it is best to envision a non-retained and a strongly retained compound during a step gradient elution, as depicted in Figure 7. A non-retained compound will always elute at the same time, irrespective of a shift of the gradient step due to different gradient delay volumes. A strongly retained peak, however, will only elute once the gradient step reaches the analyte on the column, almost independent of the absolute length of the isocratic hold prior to the gradient step. In case of the latter, any increase of GDV directly translates into a shift of the retention time.

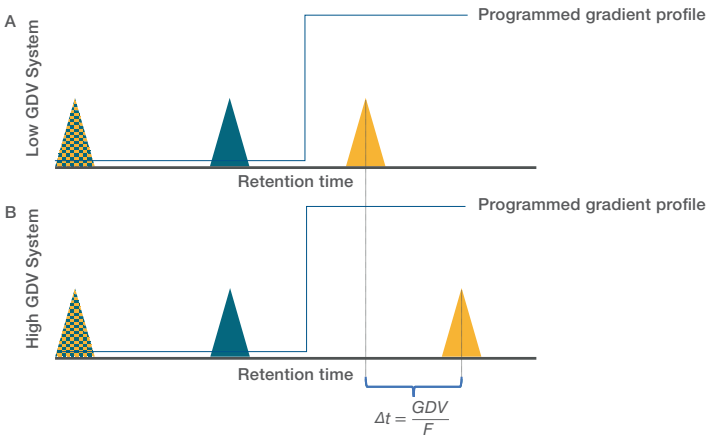

Figure 7. Impact of GDV on elution times of non-retained (blue) and strongly retained (orange) peaks during a step gradient elution (A) for a system with low GDV and (B) for a system with GDV. The retention time of the blue peak is not influenced by the GDV while the orange peak is directly shifted by the delay of the gradient (with GDV= gradient delay volume [mL], F=flow [mL/min] and Δt=retention time shift [min]).

Guidelines for setting the GDV during method transfer

If no information on the GDV difference between source and target system is available, we recommend the following method transfer procedure:

i) Replicate the chromatogram on the Vanquish Core HPLC system with an idle volume setting of 0 µL and no loop connected, using the same gradient table and settings such as data collection rate and detector signal filter (e.g. time constant) as implemented at the source instrument.

ii) Compare results between source and target systems.

iii) In case of a retention time shift, identify whether a shift towards earlier retention times is present with the Vanquish Core HPLC system.

iv) Enter a volume shift into the Chromeleon Instrument Method Editor (Figure 6) to compensate the retention time shift (in case of a not fully consistent shift you might focus on late elution peaks) by:

   a. either calculating the volume to compensate for the retention time shift with this simple equation:

$$\Delta V = (t_1 - t_2) \times F$$

   With ΔV= delta gradient delay volume [µL], t_1=retention time of peak A on source system [min], t_2=retention time of peak A on target system [min], F=flow [µL/min]

   b. or increase the volume in an iterative process until the best retention time overlap is achieved.[4]

These changes are compliant since all of the following are true:

- Compendial methods do not regulate system volumes.
- The fluidic setup of the HPLC system is not undergoing a manual change.
- Instrument parameter settings are fully trackable in the audit trail of the chromatography data system.

Compliance aspects

USP

In the method transfer for HPLC section of general chapter <621> in the USP41, modifications of the method are tolerated within limits. For the gradient delay volume (named dwell volume in the USP) it is stated that, "if adjustments are necessary, change in column packing (maintaining the same chemistry), the duration of an initial isocratic hold (when prescribed), and/or gradient delay volume adjustments are allowed."[2] This means that changes of the gradient delay volume as done by the method transfer kit are explicitly approved as a suitable tool for method transfer.

European Pharmacopeia

The European Pharmacopeia generally prefer a different design approach insofar that, "monographs preferably include an isocratic step before the start of the gradient program so that an adaptation can be made to the gradient time points to take account of differences in dwell volume between the system used for method development and that actually used. It is the user's responsibility to adapt the length of the isocratic step to the analytical equipment used."[5] Although the adaptation of the gradient delay volume is not explicitly mentioned as a tool, the effect, namely the length of the initial isocratic windows is actively encouraged as a changeable parameter. However, an approach that is based on altering the isocratic step length often fails to reflect the possible chromatographic differences due to different physical GDV, e.g. mixing effects. Therefore, if possible, physical adjustment of the GDV should be preferred.

Japanese Pharmacopeia

The Japanese Pharmacopeia can be considered liberal with respect to changing the operating conditions. A wide range of parameters "[…] may be modified within the ranges in which the liquid chromatographic system used conforms to the requirements of system suitability."[6] Adapting the GDV of a system can therefore be recommended as a suitable tool for simple method transfer.

Qualification of the Method Transfer Kit

Instrument qualification usually follows a holistic approach, testing whether an HPLC system works as intended. With Chromeleon CDS the required test sequences are generated automatically considering the used instrument configuration.

If the method transfer kit is installed and configured, Chromeleon CDS performs a duplicate of the pump gradient test. With that test, it is ensured that the flow path with and without additional loop is fully functional. Additional qualification tests besides the pump are not required as the method transfer kit does not influence autosampler, column compartment or detector performance. For more details, please refer to the *Thermo Scientific Chromeleon – Operational Qualification/ Performance Qualification for HPLC Instruments – Operating Instructions.*[7]

thermo scientific

Summary

- Gradient delay volume (GDV) is one of the most critical parameters during method transfer.
- In regulated environments, manual and not auditable changes to the HPLC system fluidics typically require a revalidation of the instrument.
- The Thermo Scientific Vanquish Core HPLC System provides a unique solution to support the method transfer from conventional HPLC instruments. The autosampler of the Vanquish Core HPLC system can freely tune the GDV of up to additional 230 µL. The optional Method Transfer Kit (P/N 6036.2100) allows to extend this range to up to 430 µL additional gradient delay volume to help transferring methods even from legacy design HPLC instruments with extensive GDV.
- Seamless integration into Chromeleon CDS and SII for Xcalibur offers fully compliant settings as the gradient delay volume is a method parameter logged in the audit trail.

References

1. Paul, C.; Grübner, M. et al. Thermo Scientific White Paper 72711: An instrument parameter guide for successful (U)HPLC method transfer, 2018. https://assets.thermofisher.com/TFS-Assets/CMD/Reference-Materials/wp-72711-lc-methodtransfer-guide-wp72711-en.pdf
2. The United States Pharmacopeial Convention, Chapter <621>, United States Pharmacopeia 41.
3. Grübner, M. Thermo Scientific Application Note 73309: Straightforward transfer of an EP method for chlorhexidine impurity analysis from an Agilent 1260 Infinity LC system to a Vanquish Core HPLC system
4. Franz, H., Wachinger, T., Schmidt, C.: DE Patent DE102015112900B4, Method of transferring a method from an initial system to a target system in liquid chromatography, 2015, Germany.
5. Chapter 2.2.46, European Pharmacopeia 7.0
6. Chapter 2.01.7, Japanese Pharmacopoeia, 17th Edition.
7. Thermo Scientific, Chromeleon Operational Qualification/Performance Qualification for HPLC instruments – Operating Instructions – Revision 9.5, 2020.

Find out more at **thermofisher.com/vanquishcore**

ThermoFisher SCIENTIFIC