Easy transfer of an EP method for chlorhexidine impurity analysis from a Shimadzu Nexera-i system to a Vanquish Core HPLC system

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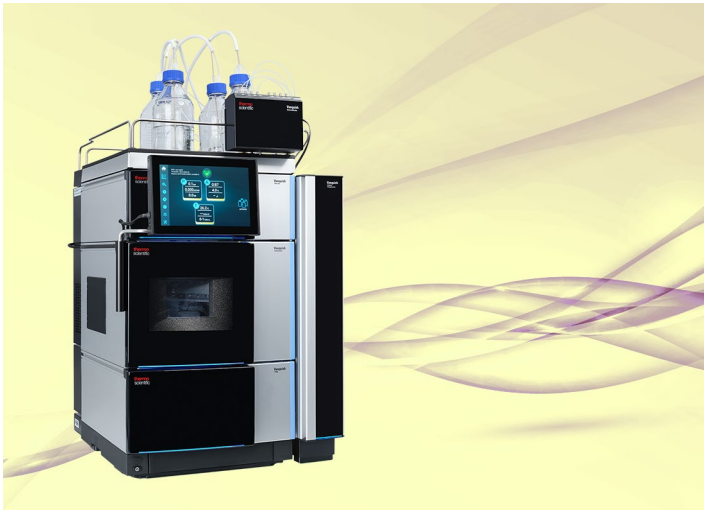

Application benefits

- Straightforward transfer of an EP monograph HPLC method from a Shimadzu™ Nexera-i™ system to a Thermo Scientific™ Vanquish™ Core Quaternary HPLC system is demonstrated.
- Advanced hardware features of the Vanquish Core HPLC system enable flexible adjustments of the overall system gradient delay volume to facilitate compliant fine-tuning during the transfer.
- Equivalent chromatographic results are obtained with the originating and receiving instrument, but improved system precision is provided by the Vanquish Core HPLC system.

Goal

To showcase the transfer of analytical HPLC methods from a Shimadzu Nexera-i system to the Vanquish Core HPLC system and highlight the easy-to-use gradient delay volume (GDV) adjustment features of the Vanquish Core HPLC system.

Introduction

Instrument-to-instrument transfer of liquid chromatographic (LC) methods is a challenging task most analytical laboratories face frequently. For example, an established application needs to be distributed over several instruments within one lab to manage the workload. In another common situation, inter-lab transfers are realized among method developing and method implementing laboratories, that is, from research and development (R&D) labs to quality control (QC) labs, or when specific tasks are outsourced to contract labs.[1] In all cases, sending and receiving instruments may differ in vendor and configuration. A third scenario is the replacement of legacy instrumentation by modern technology. In any instance, a transfer is only

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considered effective if equivalent results are obtained. The success and the required effort of a transfer depends on the robustness of the method to be transferred as well as on instrumentational deviations of the involved systems.[1] Some technical characteristics of a system, like its gradient delay volume (GDV), pump mixing mode, hydrodynamic behavior, column and eluent thermostatting options, may affect critical results like peak resolution or retention times.[2-4] The complexity of the transfer job is determined by the requirements of the chromatographer to the analytical outcome and the defined limits of acceptable deviations from the originating system. In addition, only very limited modifications of method parameters are usually accepted during a transfer to prevent the need of a time-consuming revalidation. Thus, compliant hardware features, like the unique adaptable GDV options provided by the Vanquish Core HPLC system, are the preferred tools to assist in transferring LC methods.

In the following, the HPLC method for impurity analysis of chlorhexidine digluconate given by the European Pharmacopoeia (EP) monograph[5] is transferred from a Shimadzu Nexera-i system to a Vanquish Core Quaternary HPLC system. Chlorhexidine is a common antiseptic and disinfectant, listed on the World Health Organization's (WHO) Model List of Essential Medicines.[6] It is available as an over-the-counter drug and is widely used in dental medicine and hygiene, for example, in mouthwashes and for skin disinfection purposes.

The selected Thermo Scientific™ Hypersil GOLD™ column complies with the requirement for an end-capped C18 silica column of the monograph. Although we adhered to the EP monograph, the following discussions in general are also valid for the United States Pharmacopoeia (USP) method,[7] as the analytical method, i.e., column and gradient, are identical.

Experimental details

Reagents and materials

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955-212)
- Thermo Scientific™ Pierce™ Trifluoracetic acid (TFA), LC-MS grade (P/N 85183)
- EP reference standard: Chlorhexidine for system suitability (SST) CRS batch 2 (catalog code Y0001545[8])

Instrumentation

See Table 1.

HPLC conditions

See Table 2.

Table 2. Chromatographic conditions

Parameter	Value
Column	Hypersil GOLD, 4.6 x 250 mm, 5 µm, 175 Å (P/N 25005-254630)
Mobile phase	A: 0.1% TFA in water/acetonitrile (80/20; v/v) B: 0.1% TFA in water/acetonitrile (10/90; v/v)
Flow rate	1 mL/min
Gradient	0 min – 0% B 2 min – 0% B 32 min – 20% B 37 min – 20% B 47 min – 30% B 54 min – 30% B 55 min – 0% B 62 min – 0% B
Column temp.	30 °C (forced air)
Autosampler temp.	8 °C
Detection	Vanquish Core: 254 nm, 5 Hz, response time 1 s Nexera-i: 254 nm, 4.1667 Hz, time constant 480 ms
Injection volume	7 µL
Needle wash	Off

Table 1. Instrumentation

	Shimadzu Nexera-i	Vanquish Core Quaternary
System base		System Base Vanquish Core (P/N VC-S01-A-02)
Pump	LC-2040C 3D MT; integrated system with quaternary solvent delivery, autosampler, column oven, photodiode array detector and two flow lines with UHPLC and HPLC delay volumes* *HPLC flow path used	Quaternary Pump C (P/N VC-P20-A-01)
Sampler		Split Sampler CT (P/N VC-A12-A-02)
Column compartment		Column Compartment C (P/N VC-C10-A-03)
Detector		Diode Array Detector (P/N VC-D11-A-01)
Flow cell	Fast flow cell i-series 3D (10 mm, 8 µL, 228-45618-54)	Standard (10 mm, 13 µL, P/N 6083.0510)
System accessory		Method Transfer Kit Vanquish (P/N 6036.2100)

Sample preparation

According to the monograph, 5 mg of the reference standard, which contained the active pharmaceutical ingredient (API) chlorhexidine and various impurities, were dissolved in 1 mL of mobile phase A (see Table 2).

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS), version 7.3 was used for data acquisition and analysis. Shimadzu LC drivers for Chromeleon CDS were installed for the direct control of the Nexera-i instrument.

Results and discussion

For best comparability, the following experiments were conducted with the same column, aliquots of the same sample, and the same mobile phase batch to exclude non-instrumental effects on the transfer. In deviation from the EP monograph, the injection volume was 7 µL instead of 10 µL to avoid a saturation of the main peak signal with the Nexera-i detector due to exceedance of the linearity range. Seven consecutive injections were executed with each system. Figure 1 displays the comparison of both instruments under conditions outlined in the EP monograph. The chromatogram is populated over the complete run time with peaks of the main compound, specified impurities, and unknowns not specified in the SST standard leaflet.[9] For reasons of clarity, only the peaks that exceeded a minimum peak area of 0.25 mAU·min are considered in this work.

Very similar chromatograms were generated by the Nexera-i and Vanquish Core HPLC instruments, implying a very similar chromatographic performance as can also be seen in Table 3 and Figure 2. A summary of relative retention times, experimentally obtained and provided by the EP monograph, is given in Table 3. Both instruments are in excellent accordance with each other and well aligned with the EP objectives. In Figure 2, a very good accordance in relative peak areas and peak resolutions is displayed. The retention time and peak area precisions obtained with either system are shown in Figure 3. While the relative standard deviations (RSD) of retention times is not higher than 0.05% with the Vanquish Core HPLC system, they rank up to 0.09% with the Nexera-i system. The RSD of peak areas is below 0.5% for all peaks with the Vanquish Core HPLC system. The same is true for most of the peaks with the Nexera-i instrument with some exceptions exceeding this limit.

The system suitability criteria given by the EP monograph, requiring a resolution of the impurity pair L and G of minimum 3 and a peak-to-valley ratio of impurity B of minimum 2, are easily met by either LC system with a resolution > 8 and a peak-to-valley ratio > 6. Thus, the chlorhexidine impurity LC method was successfully repeated with both systems, giving equivalent results, and its transfer could be rated as straightforward and successful.

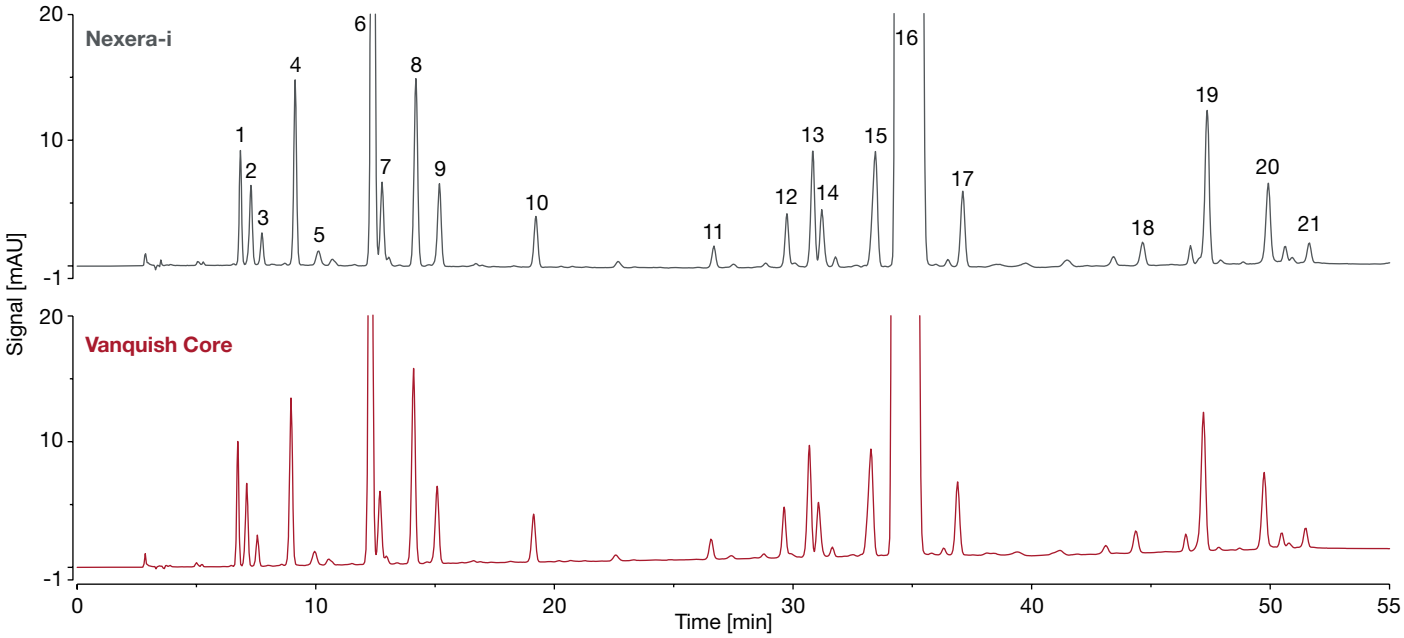

Figure 1. Transfer from Nexera-i system to Vanquish Core HPLC system according to the EP monograph for chlorhexidine gluconate; peak assignment according to impurity designation in EP monograph and standard leaflet[5, 9]

Table 3. Averaged relative retention times related to the main peak as stated in the EP monograph and from Nexera-i and Vanquish Core chromatograms (Figure 1, default settings)

Peak #	Compound	EP monograph	Nexera-i	Vanquish Core
1	Unknown 1		0.199	0.197
2	Impurity L	0.23	0.212	0.208
3	Impurity Q	0.24	0.225	0.221
4	Impurity G	0.25	0.266	0.262
5	Unknown 2		0.294	0.291
6	Impurity N	0.35	0.360	0.359
7	Impurity B	0.36	0.372	0.371
8	Impurity F	0.50	0.413	0.412
9	Unknown 3		0.442	0.441
10	Impurity A	0.60	0.559	0.559
11	Unknown 4		0.776	0.776
12	Impurity H	0.85	0.865	0.866
13	Impurity O	0.90	0.897	0.896
14	Impurity I	0.91	0.907	0.907
15	Impurity J	0.96	0.973	0.972
16	Chlorhexidine	1.00	1.000	1.000
17	Unknown 5		1.079	1.078
18	Unknown 6		1.299	1.296
19	Impurity K	1.40	1.377	1.378
20	Unknown 7		1.452	1.453
21	Unknown 8		1.502	1.503

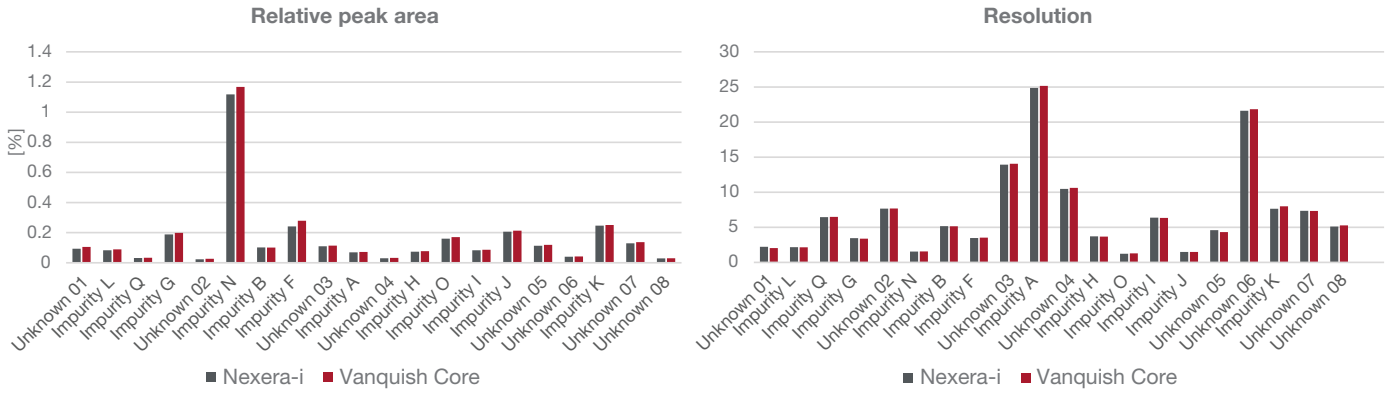

Figure 2. Chromatographic results with Nexera-i and Vanquish Core HPLC systems under conditions outlined in the EP monograph (Figure 1)

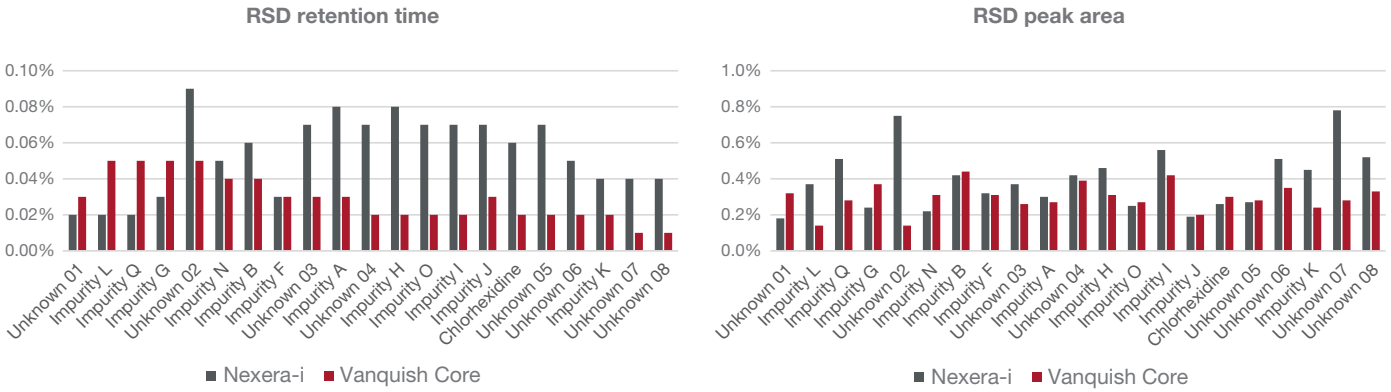

Figure 3. Relative standard deviations (RSD) of retention times and peak areas over seven injections obtained by the Nexera-i and Vanquish Core HPLC systems

However, despite the excellent fit of *relative* retention times, in a direct overlay of both chromatograms, one can observe small deviations in the *absolute* retention times with all peaks eluting slightly earlier on the Vanquish Core HPLC system (Figure 4 top). These may be the results of a slightly smaller default GDV of the Vanquish Core HPLC system compared to the HPLC flow path of the Nexera-i system. The GDV of an LC system is defined as the volume between the point of mobile phase mixing and the column head. If a closer match of absolute retention times in gradient LC methods is required, for example, to meet prescribed acceptance limits, the deviations can be compensated by a tuning of the GDV of the Vanquish Core HPLC system by two different means.

1. The idle volume setting of the autosamplers' metering device, which is the sample aspiration device, can be tuned in a range of 0–230 µL. The default setting is 25 µL.
2. An optional method transfer kit switches a 200 µL volume loop into the flow path between the pump and the autosampler.

Combining both approaches, the seamlessly tunable GDV portion of the Vanquish Core HPLC system is up to 430 µL. With this volume, retention times in gradient LC methods can be delayed to achieve a closer match with the originating system.

For the current application, the retention time deviations of the Vanquish Core HPLC system (default) compared to the Nexera-i system ranged from 0.076 to 0.26 min (Figure 5). Increasing the idle volume from the default value (25 µL) to 125 µL markedly improved the retention time match of both systems (Figure 4, bottom). Early eluting peaks were less impacted by the GDV change, as can be expected from the mixed isocratic and gradient elution mechanisms affecting these peaks. For some other peaks, the GDV increase resulted in a slight overcompensation. In total, the retention time deviations were considerably decreased as outlined in Figure 5. After the idle volume adaption, the average of absolute retention time deviations decreased from 0.132 min with the default settings to 0.051 min, demonstrating the benefit of switchable system volumes for LC method transfers. If desired, further fine-tuning can be

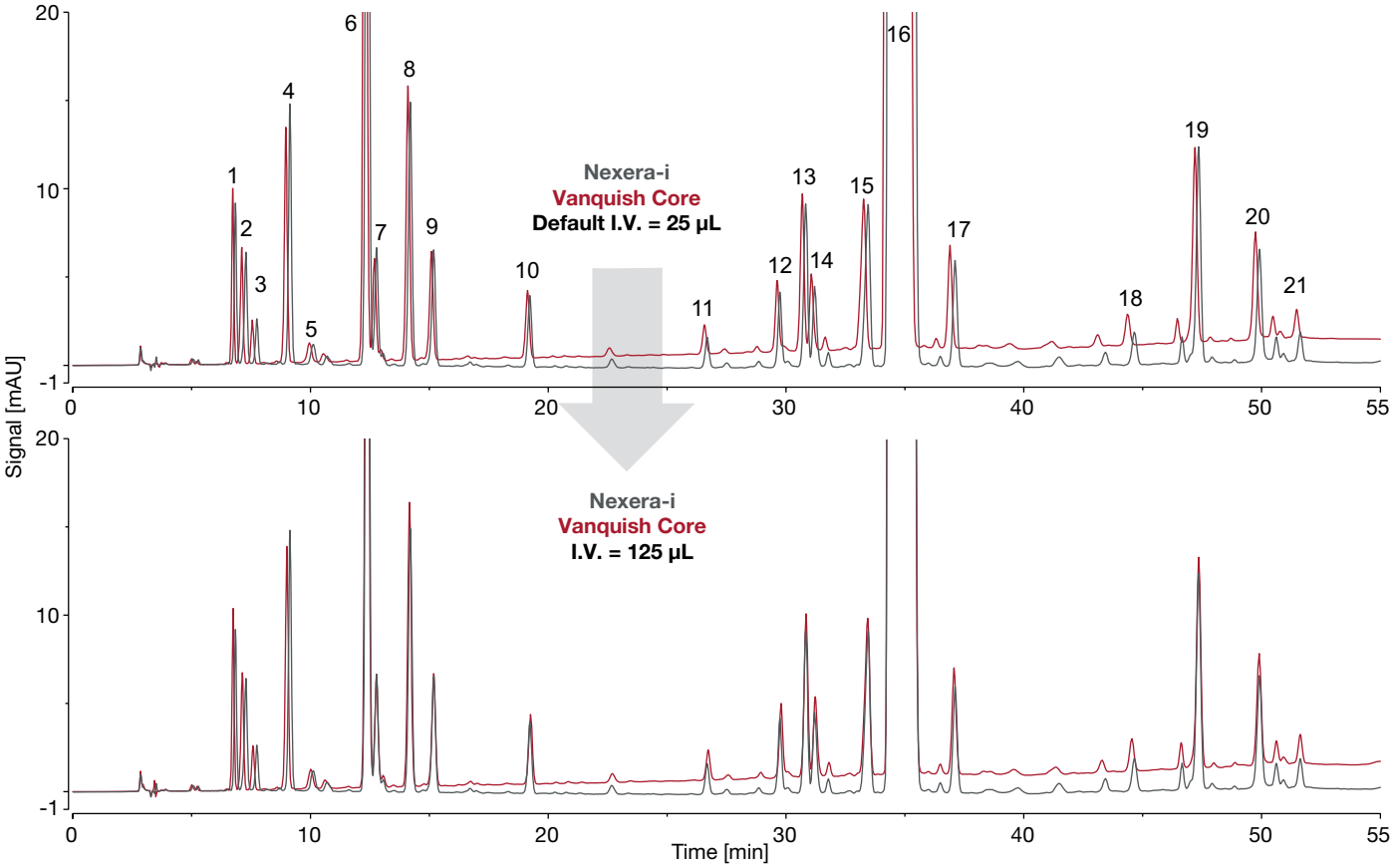

Figure 4. Retention time fine-tuning by idle volume (I.V.) adaption of the metering device in the Vanquish Core sampler

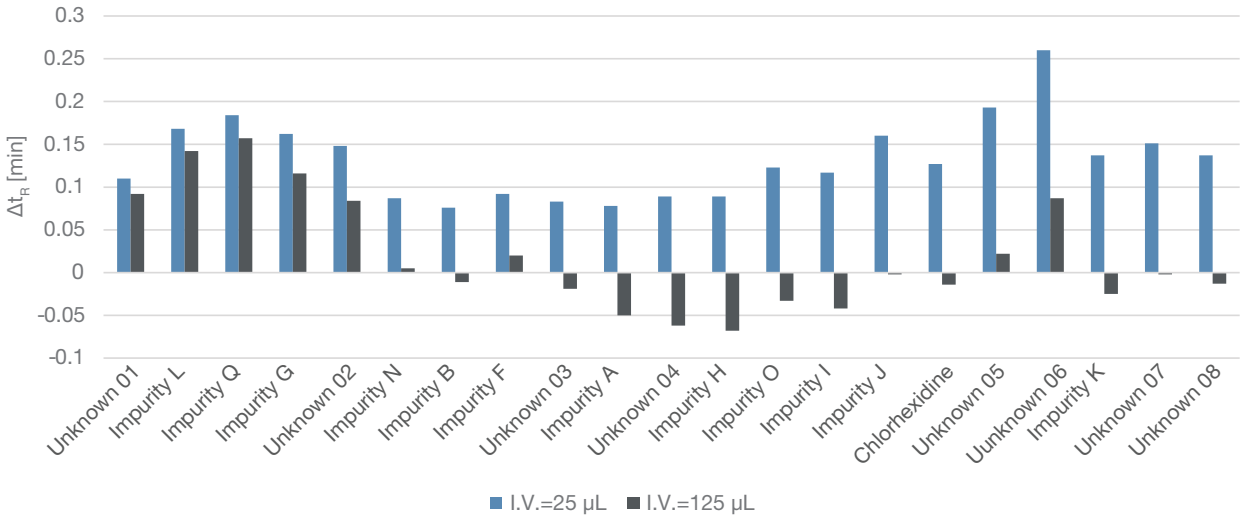

Figure 5. Retention time deviations of the Nexera-i system compared to the Vanquish Core HPLC system with an idle volume (I.V.) of 25 µL (default) and of 125 µL

done in an iterative way.[10] This flexibility is a major benefit in comparison to the method transfer concept provided by the Nexera-i system, which offers two static flow paths with two different pump mixers. In addition, the mobile phase mixing performance is not impacted by the flexible GDV tools of the Vanquish Core HPLC system.

The applied GDV changes are compliant because of the following.

- 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.

For interested readers, more details are outlined in Reference 10.

However, note that besides the GDV, other instrument-design differences may cause peak retention times to shift. Thermal effects are one common example, such as those induced by different eluent pre-heating efficiency or the absence or presence of a pre-heater.

Conclusion

- The straightforward transfer from a Shimadzu Nexera-i system to a Thermo Scientific Vanquish Core HPLC system was demonstrated for the EP method for chlorhexidine impurity analysis.
- Equivalent chromatographic outcomes were provided by the two systems with improved system precision of the Vanquish Core HPLC system.
- Small deviations of absolute retention times due to different system gradient delay volumes were easily decreased by an adjustment of the idle volume of the Vanquish Core autosampler. For further GDV increase, a Method Transfer Kit (P/N 6036.2100) is available. Either option is compliant and trackable.

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