APPLICATION NOTE 73309

Straightforward transfer of an EP method for impurity analysis of chlorhexidine from an Agilent 1260 Infinity LC system to a Vanquish Core HPLC system

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Application benefits

- Easy transfer of an EP monograph HPLC method from an Agilent® 1260 Infinity LC system to a Thermo Scientific™ Vanquish™ Core HPLC system is demonstrated.
- Enhanced hardware features of the Vanquish Core HPLC system enable flexible adjustments of the overall system gradient delay volume, which facilitates fine-tuning during the transfer.
- Equivalent chromatographic results are obtained with the originating and receiving instrument.

Goal

To showcase the transfer of analytical HPLC methods from an Agilent 1260 Infinity LC system to the Vanquish Core HPLC system and highlight the easy-to-use gradient delay volume (GDV) 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 under several scenarios. For example, an established application needs to be run by several instruments within one lab to distribute the major workload. On the other hand, 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, for example, to contract labs.[1] In both cases, the transferring and receiving laboratories' instruments can be either equivalent or different in vendor and configuration. A third scenario is the replacement of legacy instrumentation by modern technology. In any instance a transfer is only considered effective if equivalent results are obtained. The success and the required effort of such a transfer depend on multiple factors. The robustness of the method to be transferred as well as instrumentational deviations of the involved systems play an important role.[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 requirements of the chromatographer to the analytical outcome and the defined limits of acceptable deviations from the originating system determine the complexity of the transfer job. In addition, only very limited modifications of method parameters are usually accepted during a transfer to prevent the need for a time-consuming revalidation. Thus, compliant hardware features are the preferred tools to assist in transferring LC methods, for example, the adaptable GDV options provided by the Vanquish Core HPLC system.

In the following, the HPLC method for impurity analysis of chlorhexidine digluconate given by the European Pharmacopoeia (EP) monograph[5] is transferred from an Agilent 1260 Infinity LC system (1260 Infinity) to a Thermo Scientific Vanquish Core 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 well 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

Reagents and materials

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Optima™ Acetonitrile, 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 CRS batch 2, catalogue code Y0001545[8]

Sample preparation

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

Instrumentation and HPLC conditions

The instruments and the HPLC conditions used in this study are listed in Tables 1 and 2.

Data processing and software

Thermo Scientific™ Chromeleon™ Chromatography Data System software, version 7.3, was used for data acquisition and analysis.

Table 1. Instruments

	Agilent 1260 Infinity Quaternary	Vanquish Core Quaternary
System base		System Base Vanquish Core (P/N VC-S01-A-02)
Solvent storage	Solvent Cabinet (5065-9981)	Solvent Rack (P/N 6036.1350)
Pump	Quaternary Pump (G1311B)	Quaternary Pump C (P/N VC-P20-A-01)
Sampler	High Performance Autosampler (G1367E) with Autosampler Thermostat (G1330B)	Split Sampler CT (P/N VC-A12-A-02)
Column compartment	Thermostatted Column Compartment with 6 µL heat exchanger (G1316A)	Column Compartment C (P/N VC-C10-A-03) (passive pre-heater P/N 6732.0170 included in System Base ship kit)
Detector	Variable Wavelength Detector (G1314F)	Variable Wavelength Detector C (P/N VC-D40-A-01)
Flow cell	Standard (10 mm, 14 µL (G1314-60186)	Standard (10 mm, 11 µL, P/N 6077.0250)
System accessory		Method Transfer Kit Vanquish (P/N 6036.2100)

Table 2. HPLC 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 temperature	30 °C (still air)
Autosampler temp.	8 °C
Detection	254 nm, 5 Hz, response time 2 s (1260 Infinity) / 1 s (Vanquish Core)
Injection volume	10 µL
Needle wash	Off

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. 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, the focus is on all peaks that exceeded a minimum peak area of 0.3 mAU·min in the following.

Very similar chromatograms were generated by the 1260 Infinity and Vanquish Core instruments, implying a very similar chromatographic performance, as can also be seen in Figure 2 and Table 3. A summary of

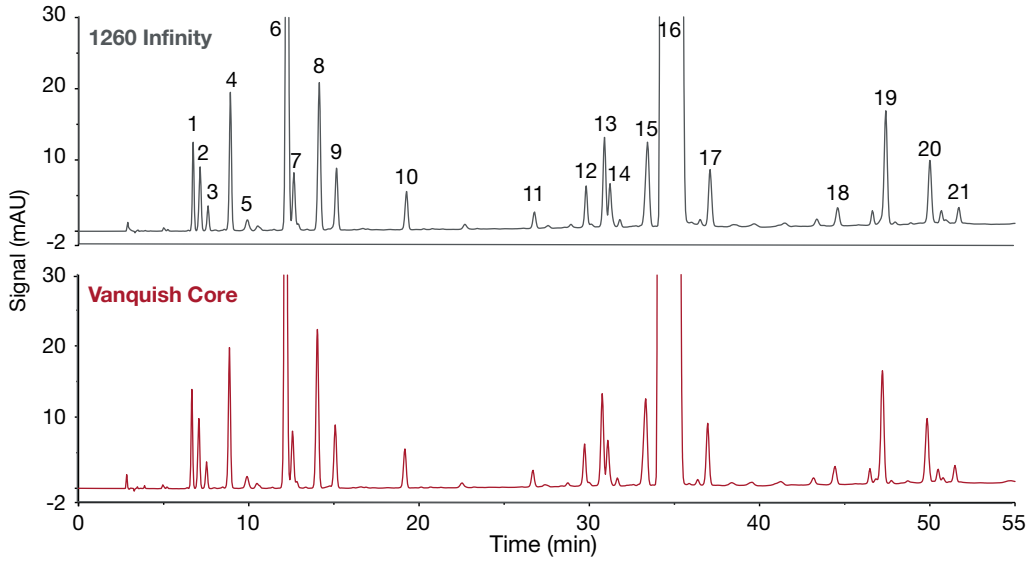

Figure 1. Transfer from 1260 Infinity system to Vanquish Core HPLC system according to the EP monograph for chlorhexidine gluconate; peak assignment according to impurity designation in EP monograph and standard leaflet[5, 9]

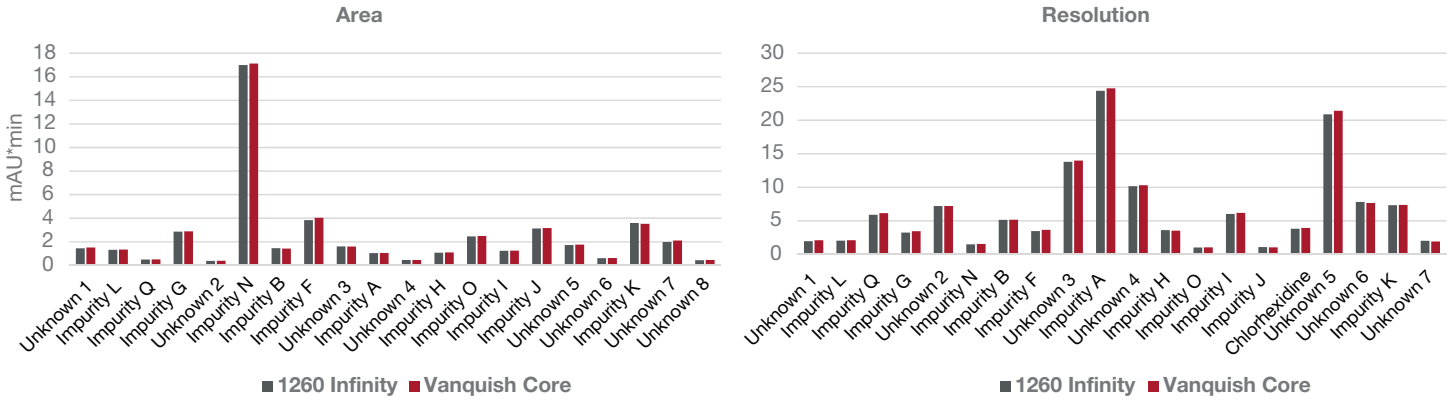

Figure 2. Chromatographic results with the 1260 Infinity and Vanquish Core HPLC systems under conditions outlined in the EP monograph (Figure 1)

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 full congruence in peak areas is displayed. Peak resolutions are in very good alignment as well, with slightly improved resolutions with the Vanquish Core HPLC system thanks to slightly narrower peaks. The retention time and peak area precisions obtained with the Vanquish Core HPLC system were excellent with a relative standard deviation of ≤0.02% for retention times and ≤0.6% for peak areas.

The system suitability criteria given by the EP monograph, claiming 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 very successful.

However despite the perfect 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 3 top). These may be the results of a slightly smaller default GDV of the Vanquish Core HPLC system compared to the 1260 Infinity. The GDV of an LC system is defined as the volume between the point of mobile phase mixing in the pump 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.

Table 3. Averaged relative retention times related to the main peak as stated in the EP monograph and from 1260 Infinity and Vanquish Core chromatograms (Figure 1, default settings)

Peak #	Compound	EP monograph	1260 Infinity	Vanquish Core
1	Unknown 1		0.196	0.195
2	Impurity L	0.23	0.208	0.207
3	Impurity Q	0.24	0.222	0.220
4	Impurity G	0.25	0.260	0.260
5	Unknown 2		0.289	0.290
6	Impurity N	0.35	0.357	0.356
7	Impurity B	0.36	0.369	0.368
8	Impurity F	0.50	0.412	0.411
9	Unknown 3		0.442	0.442
10	Impurity A	0.60	0.562	0.562
11	Unknown 4		0.781	0.782
12	Impurity H	0.85	0.870	0.871
13	Impurity O	0.90	0.901	0.901
14	Impurity I	0.91	0.911	0.911
15	Impurity J	0.96	0.975	0.976
16	Chlorhexidine	1.00	1.000	1.000
17	Unknown 5		1.083	1.083
18	Unknown 6		1.301	1.302
19	Impurity K	1.40	1.383	1.383
20	Unknown 7		1.459	1.460
21	Unknown 8		1.508	1.508

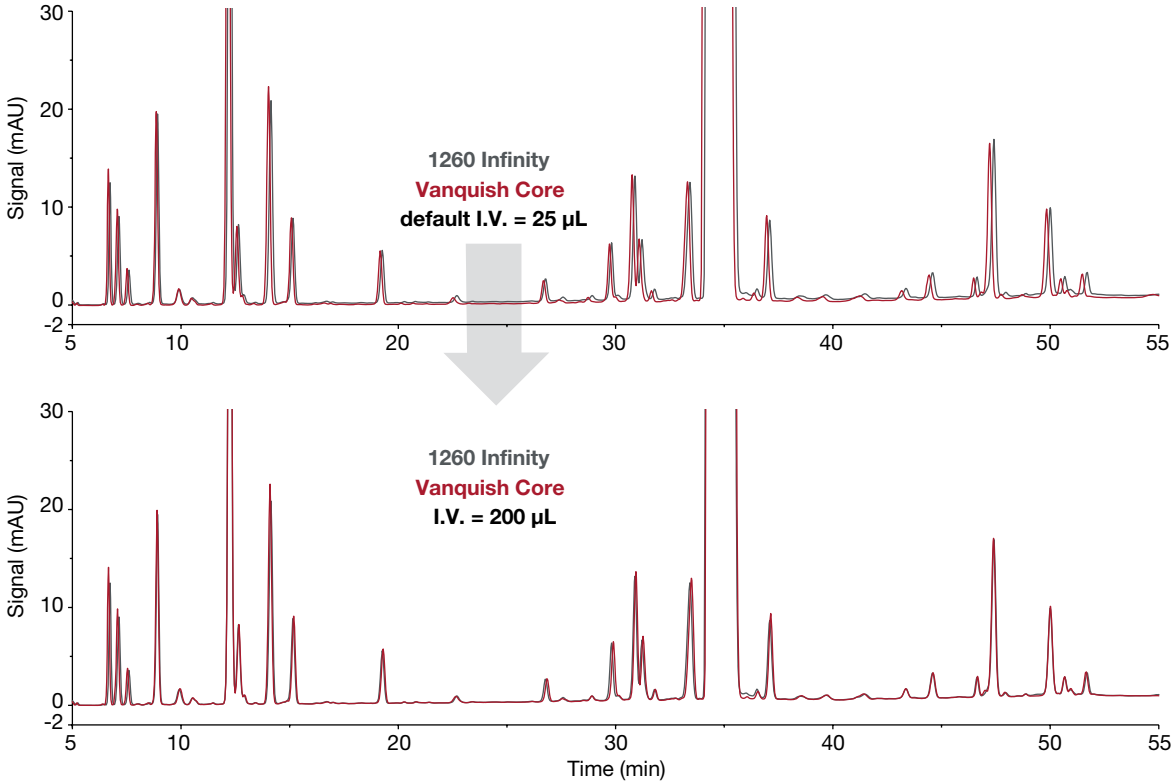

Figure 3. Retention time fine-tuning by idle volume (I.V.) adaption of the metering device in the Vanquish Core sampler

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 in order 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 1260 Infinity system ranged from 0.02 to 0.22 min, increasing roughly over the run time (Figure 4). At a flow rate of 1 mL/min, these can be translated into volume differences of 20–220 µL with a mean of 115 µL. However, early eluting peaks in gradient methods often are affected by a mixture of isocratic and gradient elution and are less affected by the GDV. Thus, for GDV adaption one would rather take into account the mean deviation of later eluting peaks in the gradient. Due to that a first estimate to increase the idle volume from the default value (25 µL) to 200 µL markedly improved the retention time match of the Vanquish Core and the 1260 Infinity systems (Figure 3, bottom). In Figure 4 the improvement is outlined for each peak. As expected, early eluting peaks were hardly impacted by the GDV increase. For some later peaks, the GDV increase overcompensated the deviations, but in total the deviations were considerably decreased, demonstrating the benefit of adaptable system volumes for LC method transfers.

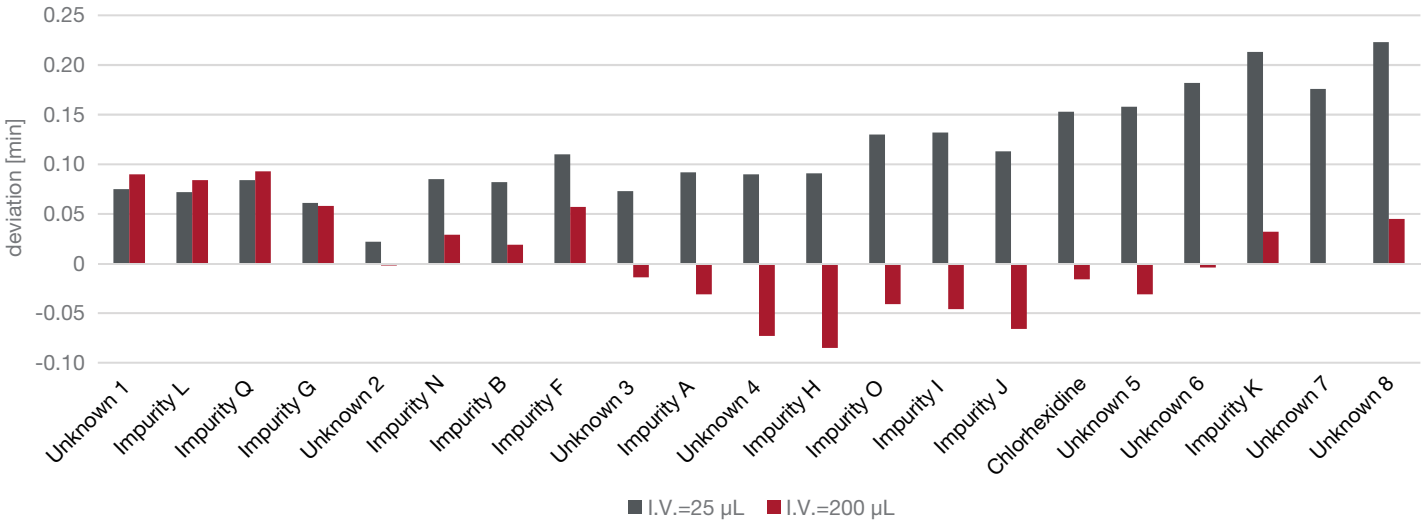

Figure 4. Retention time deviations of the 1260 Infinity system compared to the Vanquish Core HPLC system with an idle volume (I.V.) of 25 µL (default) and of 200 µL

In summary, for best retention time match, Vanquish Core HPLC system users should first replicate the chromatogram, compare with the chromatogram obtained with the source system, and then adjust the GDV of the system in an iterative process until the best retention time match is obtained.[10] The applied GDV 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.

Note that besides the GDV, other instrument-design differences may cause peak retention times to shift. One common example is thermal effects, for instance, induced by different eluent pre-heating efficiency or the absence or presence of a pre-heater.

Conclusion

- The straightforward transfer from an Agilent 1260 Infinity LC 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.
- Small deviations of absolute retention times due to different system gradient delay volumes were easily decreased by adjustments 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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References

1. Swartz, M. E.; Krull, I. Analytical Method Transfer; LCGC North America 2006, 24(11), 1204-1214, http://www.chromatographyonline.com/analytical-method-transfer-1?rel=canonical%20 (accessed Oct 29, 2019).
2. 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-method-transfer-guide-wp72711-en.pdf
3. Grübner, M. Thermo Scientific Application Note 72939: Transfer of an EP method for mebendazole from a Waters Acquity UPLC system to a Vanquish Horizon UHPLC system, 2019. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72939-uhplc-method-transfer-ep-mebendazole-an72939-en.pdf
4. Grübner, M., Paul, C., Steiner F. Thermo Scientific Application Note 72717: Method transfer of a USP derived acetaminophen assay from an Agilent 1260 Infinity system to an UltiMate 3000 SD system and a Vanquish Flex UHPLC system, 2018. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72717-lc-method-transfer-usp-acetaminophen-an72717-en.pdf
5. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.) Online, 10th edition 2018 (10.0), monograph 0658: Chlorhexidine digluconate solution
6. World Health Organization's Model List of Essential Medicines, 21th edition, June 2019, https://apps.who.int/iris/bitstream/handle/10665/325771/WHO-MVP-EMP-IAU-2019.06-eng.pdf (accessed October 24, 2019).
7. The United States Pharmacopeial Convention, United States Pharmacopeia USP42-NF37, Chlorhexidine gluconate solution monograph, 2019.
8. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).
9. Information Leaflet Ph. Eur. Reference Standard: Chlorhexidine for system suitability CRS batch 2; European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).
10. 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.

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