

Transfer of a compendial LC method for impurity analysis of chlorhexidine from a Waters Alliance HPLC system to a Vanquish Core HPLC system

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Application benefits

- Seamless transfer of an EP monograph HPLC method from a Waters™ Alliance™ HPLC system to a Thermo Scientific™ Vanquish™ Core HPLC system is demonstrated.
- Equivalent chromatographic results are obtained with both systems, but improved resolution and system repeatability are provided by the Vanquish Core HPLC system.

Goal

To demonstrate the transfer of analytical HPLC methods from a Waters Alliance HPLC system to 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 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 either 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 instrumental 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 thermostating 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 of a time-consuming revalidation.

In the following, the HPLC method for impurity analysis of chlorhexidine digluconate given by the European Pharmacopoeia (EP) monograph⁵ is transferred from a Waters Alliance HPLC system 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™ Trifluoroacetic acid (TFA), LC-MS grade (P/N 85183)
- EP reference standard: Chlorhexidine for system suitability CRS batch 2, catalogue code Y0001545⁸

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.

Table 1. Instruments

Alliance Quaternary		Vanquish Core Quaternary
System base		System Base Vanquish Core (P/N VC-S01-A-02)
Solvent storage	Bottle Tray Kit	Solvent Rack (P/N 6036.1350)
Pump		Quaternary Pump C (P/N VC-P20-A-01)
Sampler	Separation Module 2695 (no mobile phase pre-heater)	Split Sampler CT (P/N VC-A12-A-02)
Column compartment		Column Compartment C (P/N VC-C10-A-03) (passive pre-heater P/N 6732.0170 included in System Base ship kit)
Detector	Photodiode Array Detector 2996	Diode Array Detector CG (P/N VC-D11-A-01)
Flow cell	Standard (10 mm)	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 temperature	8 °C
Detection	
Wavelength	254 nm
Bandwidth	6 nm
Data collection rate	5 Hz
Filter response/ response time	1 s
Injection volume	10 µL
Needle wash	Off

*Alliance: Stroke volume = 50 µL, as recommended for flow rates of 0.531–1.230 mL/min

Data processing and software

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

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 chlorhexidine, 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.

Similar chromatograms were generated by the Alliance and Vanquish Core HPLC instruments. For all peaks the absolute retention times differed less than 4% between the systems. A summary of relative retention times, experimentally obtained and provided by the EP monograph, is given in Table 3. Both instruments are in very good accordance with each other and well aligned with the EP objectives. Good agreement on relative peak areas related to the main peak is seen in Figure 2. However, the resolution values obtained with the Vanquish Core HPLC system are usually higher than those obtained with the Alliance system; this is mainly due to narrower peaks thanks to a lower system dispersion volume of the Vanquish Core HPLC system.

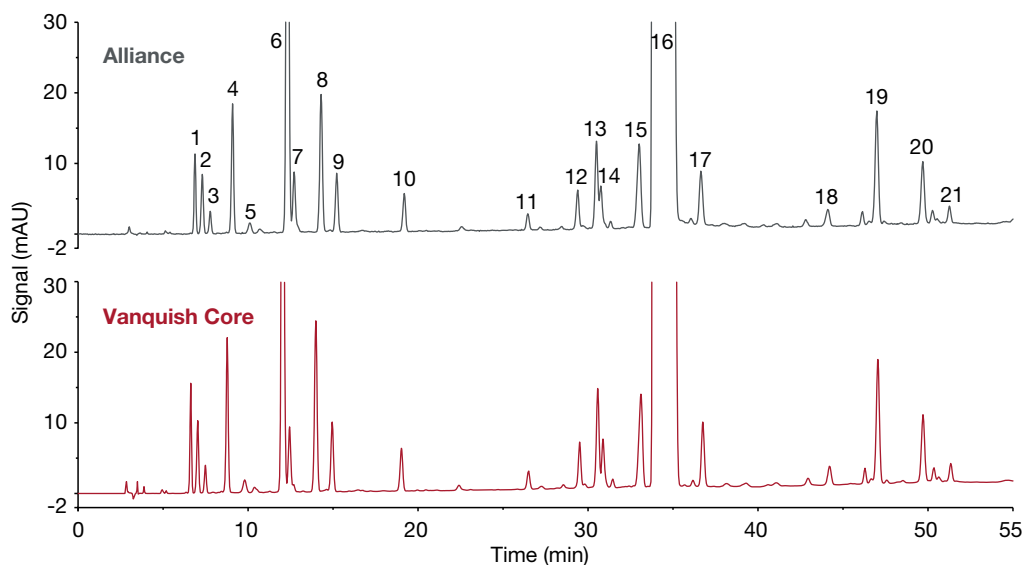


Figure 1. Transfer from Alliance system to Vanquish Core HPLC system according to the EP monograph for chlorhexidine gluconate; peak assignment according to impurity designation in EP monograph and standard leaflet^{5,9}

Table 3. Relative retention times related to the main peak as stated in the EP monograph and averaged from Alliance and Vanquish Core chromatograms (Figure 1)

Peak #	Compound	EP monograph	Alliance	Vanquish Core
1	Unknown 1		0.20	0.20
2	Impurity L	0.23	0.22	0.21
3	Impurity Q	0.24	0.23	0.22
4	Impurity G	0.25	0.27	0.26
5	Unknown 2		0.30	0.29
6	Impurity N	0.35	0.36	0.36
7	Impurity B	0.36	0.38	0.37
8	Impurity F	0.50	0.42	0.41
9	Unknown 3		0.45	0.44
10	Impurity A	0.60	0.57	0.56
11	Unknown 4		0.78	0.78
12	Impurity H	0.85	0.87	0.87
13	Impurity O	0.90	0.90	0.90
14	Impurity I	0.91	0.91	0.91
15	Impurity J	0.96	0.97	0.98
16	Chlorhexidine	1.00	1.00	1.00
17	Unknown 5		1.08	1.08
18	Unknown 6		1.30	1.31
19	Impurity K	1.40	1.39	1.39
20	Unknown 7		1.47	1.47
21	Unknown 8		1.51	1.52

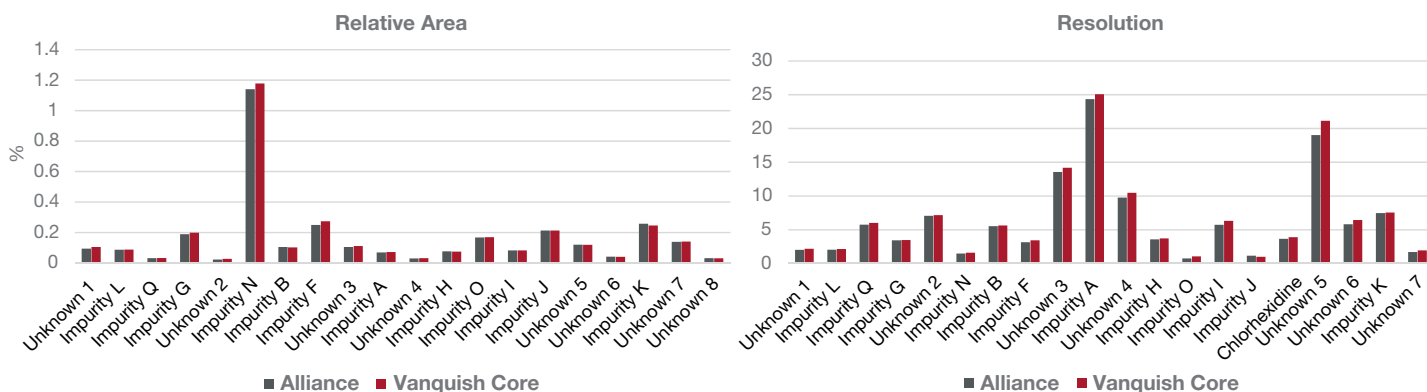


Figure 2. Chromatographic results with Alliance and Vanquish Core HPLC systems under conditions outlined in the EP monograph (Figure 1)

In addition, the repeatability of retention times and peak areas, expressed as relative standard deviations (RSD) over the seven injections, was massively improved by the Vanquish Core HPLC system as displayed in Figure 3. The RSDs of retention times for all peaks were 0.04% or lower with the Vanquish Core HPLC system, while they ranged up to 0.15% with the Alliance system. Area RSDs were 0.5% or lower for the Vanquish Core HPLC system and ranged between 0.6% and 2.8% for the Alliance. The effect is also visible in Figure 4.

The system suitability criteria given by the EP monograph, which requires a resolution of the impurity pair L and G of minimum 3 and a peak-to-valley ratio of impurity B of minimum 2, were easily met by either LC system with a resolution ~8 and a peak-to-valley ratio >5 (Alliance) and >7 (Vanquish Core). Thus, the EP method was successfully repeated with both systems without any method or hardware adaptations.

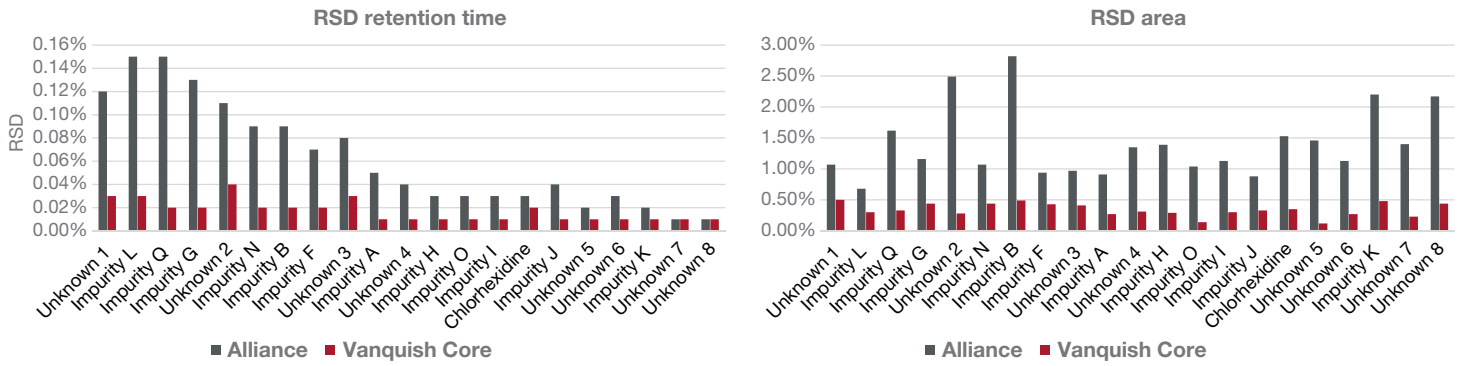


Figure 3. Relative standard deviations (RSD) of retention times and peak areas over seven injections obtained by the Alliance and Vanquish Core HPLC systems

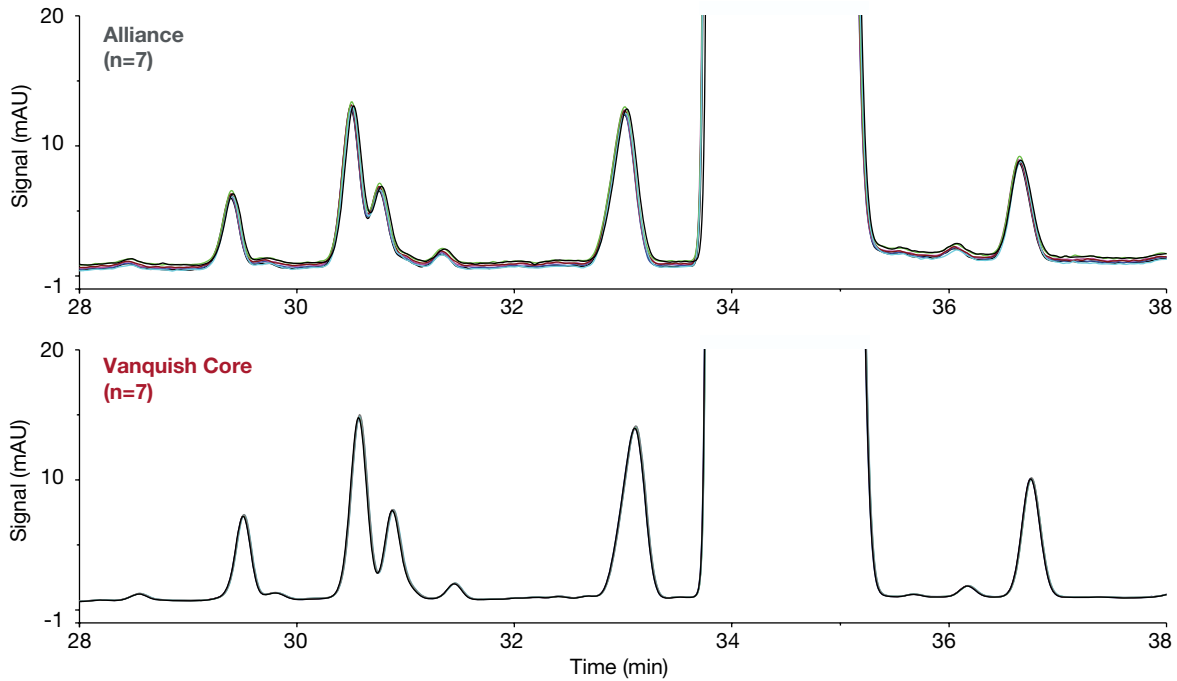


Figure 4. Overlay of seven injections by the Alliance and Vanquish Core HPLC systems, respectively, zoomed to a time range around the main peak, highlighting the improved analytical precision of the Vanquish Core HPLC system

Conclusion

- The straightforward transfer from a Waters Alliance HPLC 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. However, improved peak resolution and system repeatability was provided by the Vanquish Core HPLC system.

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