APPLICATION NOTE 73311

Seamless transfer of a compendial LC method for impurity analysis of chlorhexidine from an UltiMate 3000 Standard HPLC system to a Vanquish Core HPLC system

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Keywords: HPLC method transfer, Vanquish Core HPLC System, UltiMate 3000 Standard HPLC system, chlorhexidine, European Pharmacopoeia

Application benefits

- Straightforward transfer of an EP monograph HPLC method from a Thermo Scientific™ UltiMate™ 3000 Standard HPLC system to a Thermo Scientific™ Vanquish™ Core HPLC system is demonstrated.
- Equivalent chromatographic results are obtained with both systems, but an improved resolution and retention time stability are provided by the Vanquish Core HPLC system.

Goal

To demonstrate the transfer of analytical HPLC methods from an UltiMate 3000 Standard HPLC system to a 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 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 of a time-consuming revalidation.

In the following, the HPLC method for impurity analysis of chlorhexidine digluconate given by the European Pharmacopoeia (EP) monograph[5] is transferred from an UltiMate 3000 Standard HPLC system (UltiMate 3000 SD) to a Vanquish Core HPLC system (Vanquish Core). 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 (SST) CRS batch 2, catalogue code Y0001545[8]

Sample preparation

According to the monograph, 5 mg of the reference standard, which contained the chlorhexidine and various impurities, were solved 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

	UltiMate 3000 SD Quaternary	Vanquish Core Quaternary
System base		System Base Vanquish Core (P/N VC-S01-A-02)
Solvent storage	Solvent Rack SR-3000 (P/N 5035.9200)	Solvent Rack (P/N 6036.1350)
Pump	Quaternary Pump LPG-3400SD (P/N 5040.0031)	Quaternary Pump C (P/N VC-P20-A-01)
Sampler	Well Plate Autosampler WPS-3000TSL Analytical (P/N 5822.0020)	Split Sampler CT (P/N VC-A12-A-02)
Column compartment	Thermostatted Column Compartment TCC-3000SD (P/N 5730.0010) (passive pre-heater not included in default configuration)	Column Compartment C (P/N VC-C10-A-03) (passive pre-heater P/N 6732.0170 included in System Base ship kit)
Detector	Diode Array Detector DAD-3000 (P/N 5082.0010)	Diode Array Detector CG (P/N VC-D11-A-01)
Flow cell	Analytical (10 mm, 13 µL (P/N 6082.0100)	Standard (10 mm, 13 µL, P/N 6083.0510)
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 (forced air)
Autosampler temperature	8 °C
Detection Wavelength Bandwidth Data collection rate Filter response/ response time	 254 nm 4 nm 5 Hz 1 s
Injection volume	10 µL
Needle wash	Off

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. Six consecutive injections were executed with each system. Figure 1 displays the comparison of both instruments in their default configurations under conditions as outlined in the EP monograph. The chromatogram is populated over the complete run time with peaks of the main compound 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.

The UltiMate 3000 SD and Vanquish Core HPLC systems differ in one major feature in their default configurations: the absence (UltiMate 3000 SD) and presence (Vanquish Core) of a passive eluent pre-heater. Nevertheless, very similar chromatograms were generated by both instruments, implying a very similar chromatographic performance. A summary of relative retention times, experimentally obtained and provided by the EP monograph, is given in Table 3. The instruments are in excellent accordance with each other and well aligned with the EP objectives. For all peaks, the absolute retention times differed less than 1.2% from system to system. In addition, full congruence in peak areas is seen in Figure 2, with less than 3% deviation between the systems for each peak.

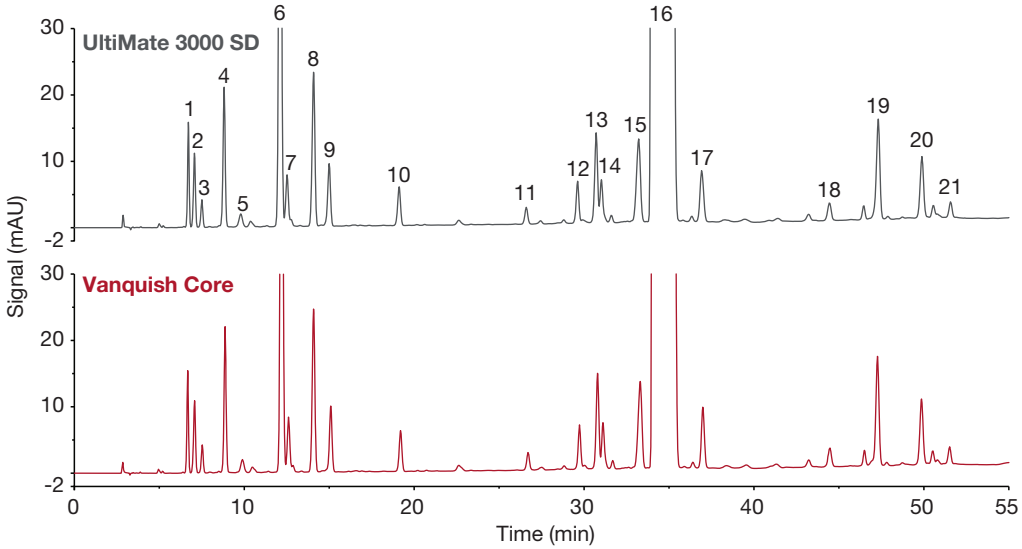

Figure 1. Transfer from UltiMate 3000 SD system to Vanquish Core HPLC system according to the EP monograph for chlorhexidine gluconate; peak assignment according to impurity designation in EP monograph and standard leaflet[5, 9]

Table 3. Relative retention times related to the main peak as stated in the EP monograph and averaged from UltiMate 3000 SD and Vanquish Core chromatograms (Figure 1)

Peak #	Compound	EP monograph	UltiMate 3000 SD	Vanquish Core
1	Unknown 1		0.20	0.20
2	Impurity L	0.23	0.21	0.21
3	Impurity Q	0.24	0.22	0.22
4	Impurity G	0.25	0.26	0.26
5	Unknown 2		0.29	0.29
6	Impurity N	0.35	0.36	0.36
7	Impurity B	0.36	0.37	0.37
8	Impurity F	0.50	0.41	0.41
9	Unknown 3		0.44	0.44
10	Impurity A	0.60	0.56	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	0.98	0.98
16	Chlorhexidine	1.00	1.00	1.00
17	Unknown 5		1.08	1.09
18	Unknown 6		1.31	1.30
19	Impurity K	1.40	1.39	1.39
20	Unknown 7		1.47	1.46
21	Unknown 8		1.52	1.51

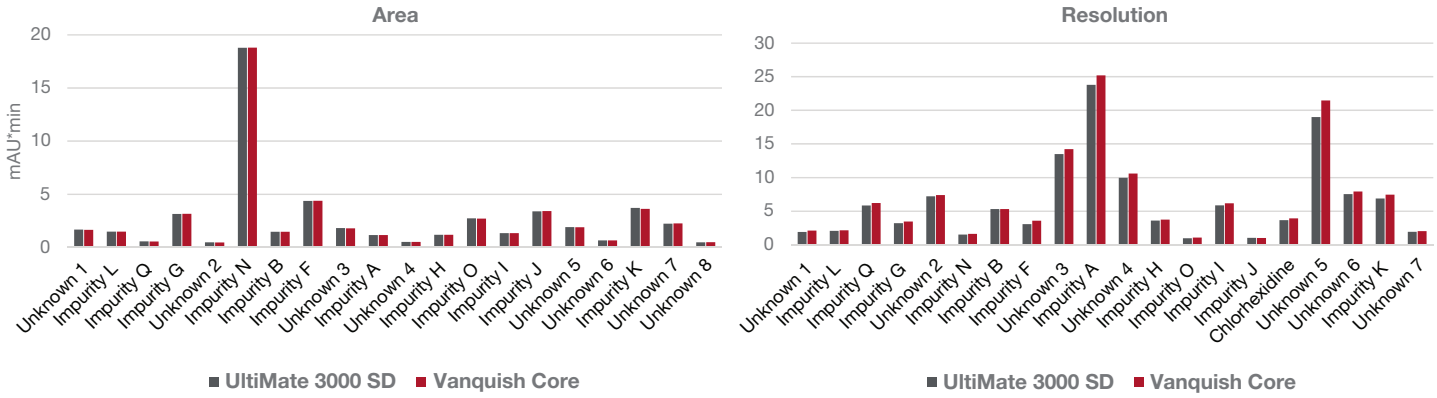

Figure 2. Chromatographic results with UltiMate 3000 SD and Vanquish Core HPLC systems under conditions outlined in the EP monograph (Figure 1)

However, the resolutions obtained by the Vanquish Core HPLC system are usually slightly higher than those obtained by the UltiMate 3000 SD system; this is mainly due to smaller peak widths caused by a lower dispersion volume of the Vanquish Core HPLC system. Both instruments provided equivalent repeatability of peak areas, expressed as relative standard deviations (RSD) over the six injections in Figure 3. Usually the RSD of peak areas was ≤0.5% with just one exception. The repeatability of retention times, however, was considerably improved by the Vanquish Core HPLC system as displayed in Figure 3. The RSDs of retention times for all peaks were lower than 0.03% with the Vanquish Core HPLC system, while they ranged up to 0.16% with the UltiMate 3000 SD system. The effect is also visualized in Figure 4.

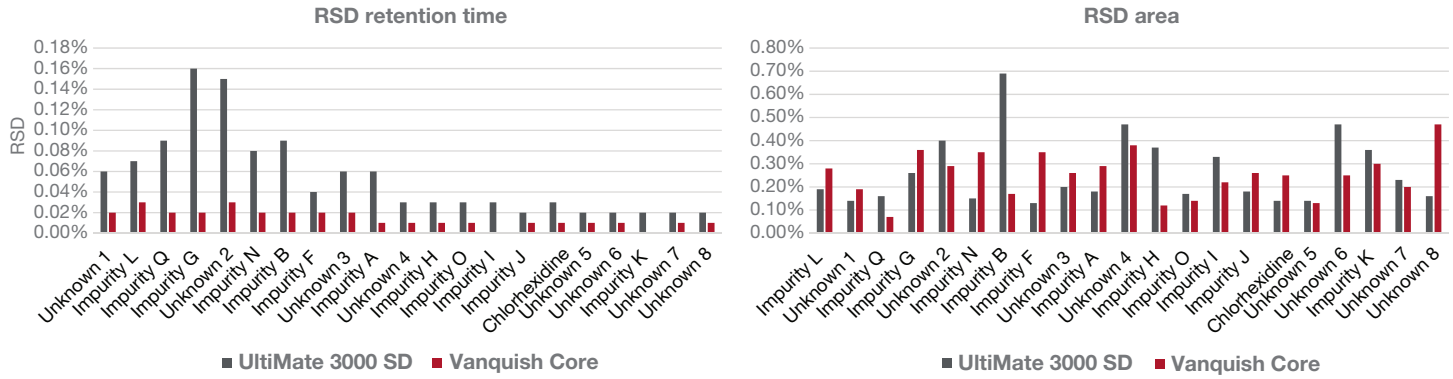

Figure 3. Relative standard deviations (RSD) of retention times and peak areas over seven injections obtained by the UltiMate 3000 SD and Vanquish Core HPLC systems

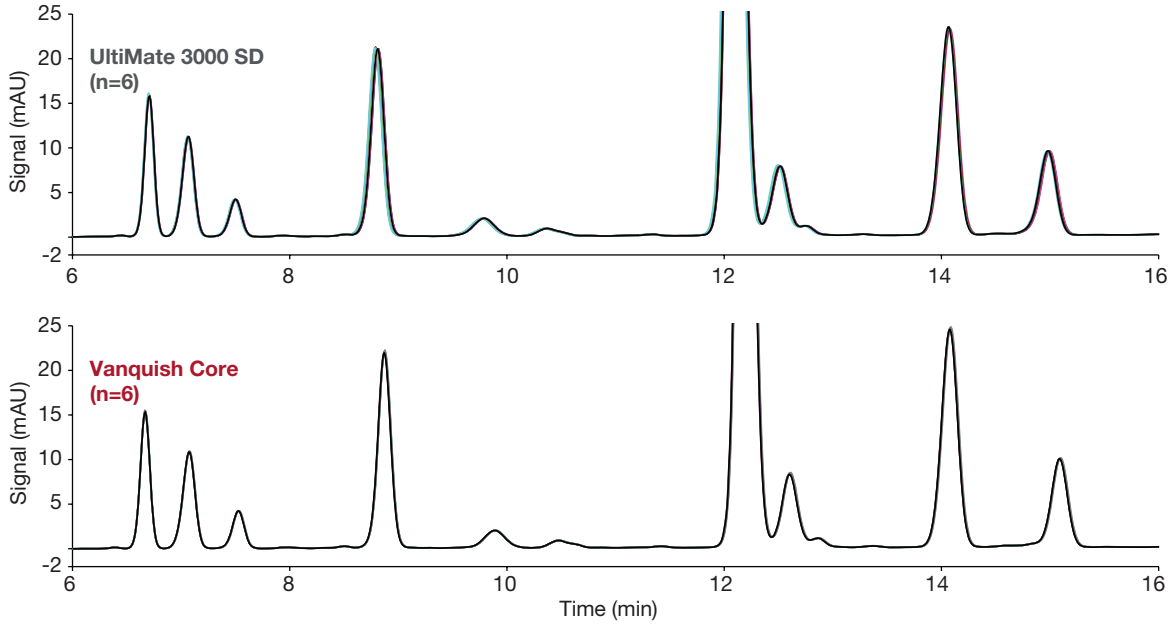

Figure 4. Overlay of six injections respectively by the UltiMate 3000 SD and Vanquish Core HPLC systems zoomed to a 10 min time segment, highlighting the improved analytical precision of the Vanquish Core HPLC system

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, were easily met by either LC system with a resolution ~8 and a peak-to-valley ratio ~6 (UltiMate 3000 SD) and >7 (Vanquish Core). Thus, the EP method was successfully repeated with both systems and the transfer was successfully conducted without any method or hardware adaptions.

Conclusion

- The seamless transfer from a Thermo Scientific UltiMate 3000 Standard 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 retention time repeatability was provided by the Vanquish Core HPLC system.

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