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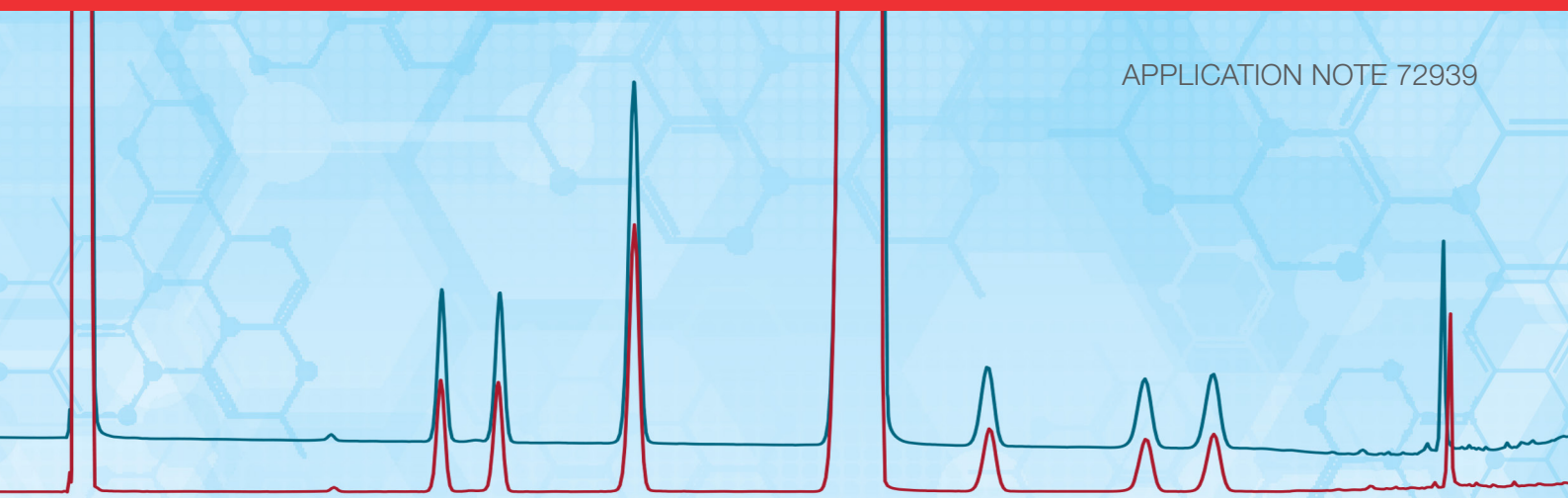

Transfer of an EP method for mebendazole from a Waters Acquity UPLC system to a Vanquish Horizon UHPLC system

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Keywords

HPLC method transfer, Vanquish Horizon UHPLC system, Waters Acquity UPLC system, mebendazole, European Pharmacopoeia

Goal

To showcase the transfer of analytical HPLC methods from a Waters Acquity UPLC system to the Vanquish platform and highlight the impact of column thermostatting.

Application benefits

- Straightforward transfer of an EP monograph HPLC method from a Waters™ Acquity™ UPLC system to a Thermo Scientific™ Vanquish™ Horizon UHPLC system is demonstrated.
- During method transfer flexible thermostatting options provided by the Vanquish platform help to mimic the actual conditions at the original system.
- Substantial time and solvent savings are obtainable by speeding up conventional HPLC methods to UHPLC conditions without sacrificing chromatographic performance.

Introduction

Instrument-to-instrument transfer of liquid chromatographic (LC) methods is a challenging but frequently occurring task in most analytical laboratories. Within one lab, applications often need to be established at several instruments due to varying instrument availability and numbers of required analyses. Inter-lab transfers are commonly executed among method developing and method implementing laboratories.[1,2] In both cases, sending and receiving units can either equal or differ in configuration and vendor. Additionally, the replacement of legacy instruments by modern ones requires thorough method transfer, which is only effective if equivalent results are obtained.

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The success and required effort of a method transfer depend on multiple factors. The robustness of the transferred method plays an important role, especially when instrumentational variations affect the analysis.[1,2] It is well known that changing the pump type (low-pressure or high-pressure mixing) can have an impact on the separation, and also the gradient delay volume (GDV; hold-up volume from solvent mixing point at pump to column head) of a system is a commonly considered factor during method transfer.[3-5] However, other impacts, such as column thermostatting, are frequently underestimated but have a strong influence as will be shown in this application note. Furthermore, the claims of the chromatographer to the analytical outcome and the defined limits of acceptable deviations from the originating system add to the complexity of the transfer job.

In the following, an HPLC method for mebendazole impurity analysis according to the European Pharmacopoeia (EP) monograph[6] is transferred from a Waters Acquity UPLC system to a Thermo Scientific Vanquish Horizon UHPLC system. Mebendazole is a well-established anthelminthic drug for the treatment of various parasitic worm infestations. It is available as a generic drug and is listed on the World Health Organization's (WHO) Model List of Essential Medicines.[7]

The selected column is a Thermo Scientific™ Hypersil GOLD™ column that well complies with the requirement for a base-deactivated C18 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,[8] as the analytical method, i.e. column and gradient, are identical. The EP and USP monographs mainly differ in the preparation of sample solutions.

Experimental

Reagents and materials

- Deionized water, 18.2 MΩ·cm resistivity or higher
- Fisher Scientific™ Acetonitrile, Optima™ LC/MS grade (P/N A955-212)
- Fisher Scientific N,N-Dimethylformamide, Acros Organics™, ACS reagent (P/N 10567942)
- Fisher Scientific Ammonium acetate, Optima LC/MS grade (P/N A115-50)
- EP reference standard: Mebendazole for system suitability CRS batch 1, catalogue code Y0000144[9]

Sample preparation

According to the monograph, 5 mg of the reference standard, which contained the active pharmaceutical ingredient (API) mebendazole and the impurities A, B, C, D, E, F, and G, were dissolved in 5 mL dimethylformamide (DMF).

Instrumentation

The instruments listed in Table 1 were used In the current study.

Table 1. Instrumentation standard configurations

	Acquity UPLC system	Vanquish Horizon UHPLC system
Pump	Binary Solvent Manager	Binary Pump H (P/N VH-P10-A)
Autosampler	Sample Manager	Split Sampler HT (P/N VH-A-10-A)
Sample Loop	10 µL	Default 25 µL (V=50 µL, P/N 6850.1911) or 10 µL (V=23 µL, P/N 6850.1915)
Column Compartment	High Temperature Column Heater	Column Compartment H (P/N VH-C10-A)
Detector	Tunable Ultraviolet Detector	Variable Wavelength Detector F (P/N VF-D40-A)
Flow Cell	Analytical (10 mm, 500 nL)	Semi-micro (7 mm, 2.5 µL, P/N 6077.0360)

HPLC conditions

Column:	Hypersil GOLD, 4.6 × 100 mm, 3 µm, 175 Å (P/N 25003-104630)
Mobile Phase:	A: 7.5 g/L Ammonium acetate in water B: Acetonitrile
Flow Rate:	1.2 mL/min
Gradient:	0 min – 20% B 15 min – 30% B 20 min – 90% B 25 min – 90% B 25.1 min – 20% B 30 min – 20% B
Column Temp.:	40 °C (still air) with eluent pre-heating or as outlined elsewhere
Autosampler Temp.:	10 °C
Detection:	250 nm Vanquish Horizon: 10 Hz data collection rate, 0.5 s response time Acquity: 10 Hz data collection rate, normal filter time constant (0.2 s)
Injection Volume:	5 µL
Needle Wash:	Vanquish Horizon: Off Acquity: 200 µL Acetonitrile and 600 µL starting mobile phase

UHPLC conditions

Column:	Hypersil GOLD, 2.1 × 50 mm, 1.9 µm, 175 Å (P/N 25002-052130)
Mobile Phase:	A: 7.5 g/L Ammonium acetate in water B: Acetonitrile
Flow Rate:	0.8 mL/min
Gradient:	0 min – 20% B 2.35 min – 30% B 3.13 min – 90% B 3.91 min – 90% B 3.93 min – 20% B 4.7 min – 20% B
Column Temp.:	40 °C (still air) with eluent pre-heating or as outlined elsewhere
Autosampler Temp.:	10 °C
Detection:	250 nm Vanquish Horizon: 50 Hz data collection rate, 0.1 s response time Acquity: 40 Hz data collection rate, normal filter time constant (0.05 s)
Injection Volume:	1 µL
Needle Wash:	Vanquish Horizon: Off Acquity: 200 µL Acetonitrile and 600 µL starting mobile phase

Data processing and software

Thermo Scientific™ Chromeleon™ Software 7.2.9 Chromatography Data System (CDS) was used for data acquisition and analysis.

Results and discussion

Before a method transfer is started it is meaningful to take an in-depth review of the instrumentational differences of both systems and what kind of chromatographical differences could be expected from them. For example, in the current case the light paths of the detector flow cells differ by around 30%, so lower absolute peak heights and areas can be presumed for the Vanquish Horizon system. Additionally, instead of the passive mobile phase preheating that is accomplished by the column stabilizer assembly in the Acquity system, the Vanquish Horizon system has an active preheater available in the standard configuration, which may induce different thermal conditions at the column head. Furthermore, the Acquity system utilizes a pulled-loop Sample Manager with a 10 µL sample loop, while the Vanquish Horizon system autosampler is a split-loop design where sample loop with a default total loop volume of 50 µL and needle are part of the flow path. These sample loop differences as well as differences in pump mixing volumes translate into different GDVs, which may result in retention time shifts. Finally, differences in the pump design and flow control may cause minor deviations in the elution pattern.

Transfer of EP method for mebendazole impurity analysis

For best comparability, all evaluations were conducted with the same column and sample and with five repeated injections. The chromatograms in Figure 1 display the comparison of both instruments under conditions as outlined in the EP monograph, and Table 2, Table 3, and Figure 2 summarize the chromatographic results. The relative retention times were well aligned with the EP monograph and in very good accordance with each other (see Table 3). In Figure 2 a full agreement on relative areas of impurity peaks is seen. The relative standard deviation (%RSD) of peak areas was not higher than 0.4% for the Vanquish Horizon system and equivalent or better than for the Acquity system. The signal-to-noise ratios of all impurity peaks were slightly higher for the Vanquish Horizon system despite the smaller light path length of the detector flow cell. Additionally, narrower peaks were produced by the Vanquish Horizon system and resolutions improved (Figure 2). The EP system suitability criterion of a peak-to-valley ratio of minimum 4 for the API and impurity D peaks was easily met by either system. Taking all of this together most chromatographers would rate this as a very successful method transfer without any special intervention and would conclude the method transfer evaluation.

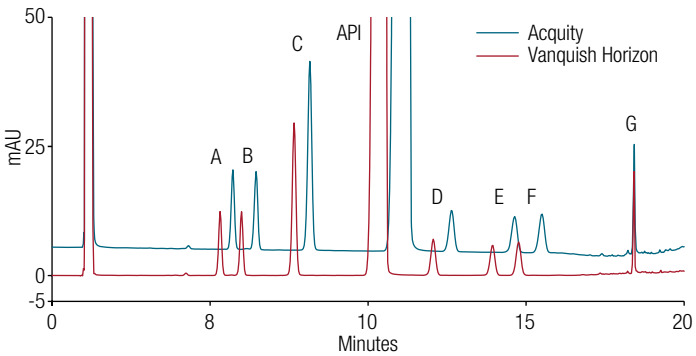

Figure 1. Transfer from Acquity system to Vanquish Horizon system according to EP monograph for mebendazole; peak assignment according to impurity designation in EP monograph

However, the deviations in absolute retention times (t_R) observed in Figure 1 and Table 2 might raise doubts or even pose an issue, if they exceed maximum acceptance limits defined in a certain lab. Thus, further elucidation is presented in a later section.

Table 2. Averaged absolute retention times in minutes over five injections for Acquity and Vanquish Horizon systems under conditions as outlined in EP monograph (Figure 1) and % deviation

Compound	Acquity	Vanquish Horizon
Impurity A	5.718	5.320 (Δ-7.0%)
Impurity B	6.454	5.996 (Δ-7.1%)
Impurity C	8.155	7.660 (Δ-6.1%)
Mebendazole (API)	11.225	10.526 (Δ-6.2%)
Impurity D	12.641	12.062 (Δ-4.6%)
Impurity E	14.635	13.938 (Δ-4.8%)
Impurity F	15.500	14.761 (Δ-4.8%)
Impurity G	18.425	18.417 (Δ-0.0%)

Table 3. Averaged relative retention times related to the API peak as stated in the EP monograph and from Acquity and Vanquish Horizon chromatograms (Figure 1)

Compound	EP monograph	Acquity	Vanquish Horizon
Impurity A	0.4	0.51	0.51
Impurity B	0.5	0.58	0.57
Impurity C	0.7	0.73	0.73
Mebendazole (API)	1.0	1.00	1.00
Impurity D	1.1	1.13	1.15
Impurity E	1.3	1.30	1.32
Impurity F	1.4	1.38	1.40
Impurity G	1.6	1.64	1.75

Figure 2. Chromatographic results with Acquity and Vanquish Horizon systems under conditions as outlined in the EP monograph (Figure 1); noise calculated from current chromatogram 2.0–3.0 min

Method scaling to UHPLC conditions

The fact that both systems utilized in the current study were designed to perform ultra-high-performance separations prompted the translation of the classical HPLC method for mebendazole analysis into a fast UHPLC method. Although currently EP and USP still refrain from permitting method scaling it is a worthwhile objective. A re-validation is required after such a translation but is usually justified by substantial savings in analysis time, solvent consumption, and costs. The speed-up method was easily calculated for the selected column dimension (2.1 × 50 mm, particle size 1.9 µm) by the new Thermo Fisher Scientific online tool.[10] Working with the Chromeleon CDS also offers the UHPLC speed-up calculator in the instrument method editing view. Savings of 90% eluent use and 84% run time resulted from the new method as depicted in Figure 3, which also shows the enormous gain in throughput. To reduce the GDV difference of both systems, the Vanquish Horizon system was now operated with a smaller sample loop (10 µL). The injection volume was set to 1 µL instead of the calculated 0.59 µL to deviate less from the Waters recommendation to use only injection volumes of 2–7.5 µL with the installed loop at the Acquity system.

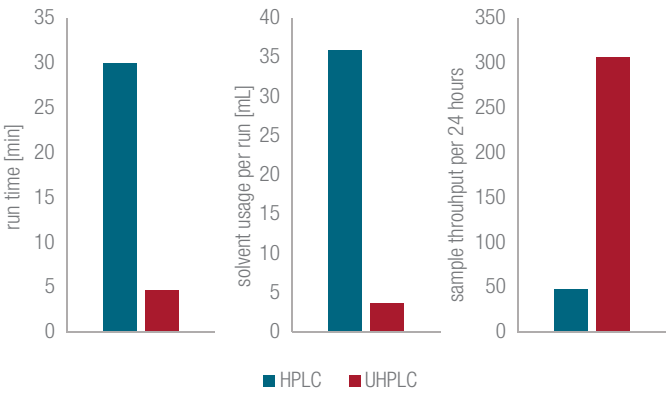

Figure 3. Comparison of EP HPLC method and speed-up UHPLC method with respect to analysis time, solvent consumption, and throughput

The obtained chromatograms are displayed in Figure 4. The relative retention times (related to the API peak) were in very good agreement with the original HPLC method; peak resolutions were only slightly decreased but never below 2.3, ensuring baseline separation of all peaks (Figure 5). The relative peak areas

were well in line with the HPLC method results for the impurities A–F, which are structurally closely related to the API (Figure 5). In contrast, relative areas for impurity G were lower than under HPLC conditions as the dimer of the API impurity G structurally differs substantially from the other compounds. Hence a deviant UV response curve was expectable, resulting in different area ratios for different injection amounts.

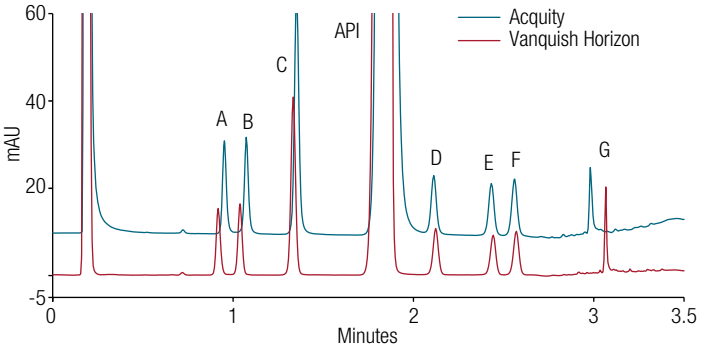

Figure 4. Chromatograms of down-scaled UHPLC method with Acquity and Vanquish Horizon systems; peak assignment according to impurity designation in EP monograph

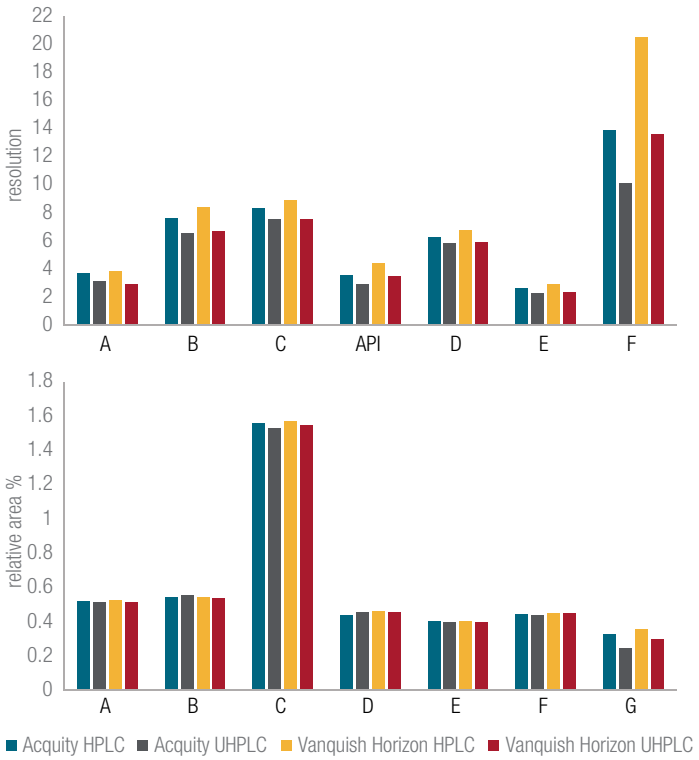

Figure 5. Peak resolution and relative peak areas for Acquity and Vanquish Horizon systems under HPLC conditions as outlined in the monograph and UHPLC conditions

The %RSD of peak areas was below 0.5% for the Vanquish Horizon system (Figure 6) and higher for the Acquity system; however, it should be noted that it was used outside its recommended injection volume range. Peak widths and S/N ratios were similar with both systems (Figure 6).

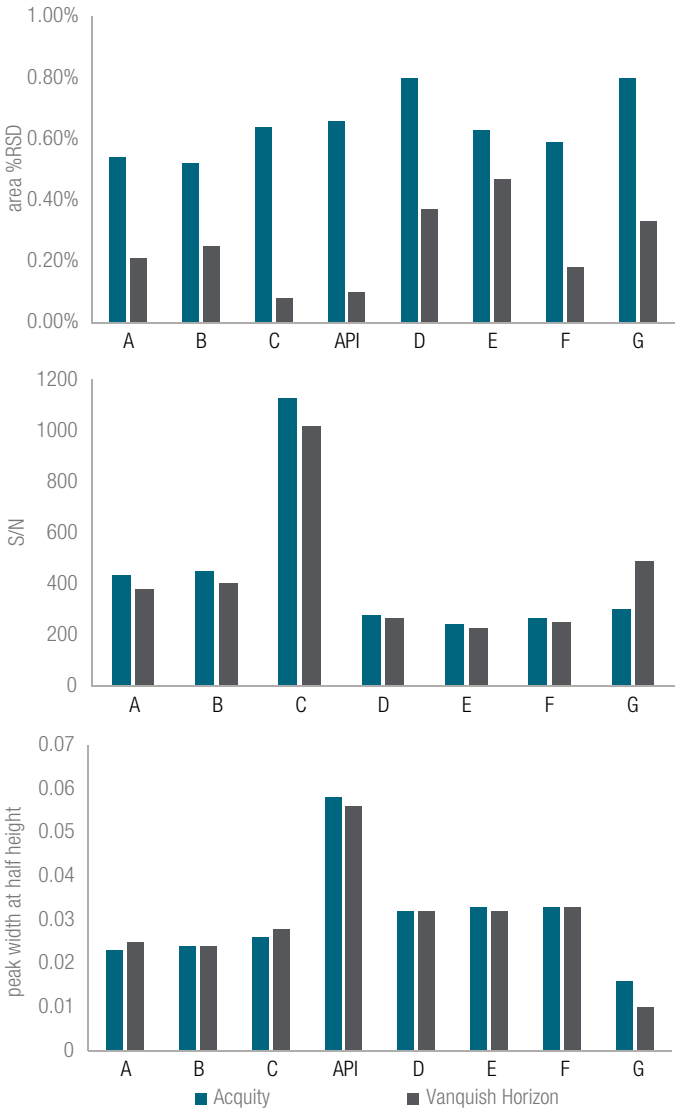

Figure 6. Chromatographic results with Acquity and Vanquish Horizon systems under UHPLC conditions (Figure 4); noise calculated from current chromatogram 0.45–0.60 min

Temperature effects on absolute retention times

As visible in Figure 1 and Figure 4, distinct deviations in absolute t_R were obtained with the Acquity and the Vanquish Horizon systems for the HPLC as well as the UHPLC methods. For the HPLC conditions, these are up to -7% for the Vanquish Horizon system (Table 1) and ranged from -3.9% to 2.9% for the UHPLC method.

At first glance the early elution of the Vanquish Horizon system under HPLC conditions is surprising. On the one hand, because of the slightly larger GDV one would rather expect a later elution compared to the Acquity system. On the other hand, the deviations were too large to be explained just by GDV differences, as they would imply GDV differences of more than 500 µL for two systems that actually exhibit total GDVs of less than 200 µL. Thus, column thermostatting came into focus. To exclude such effects, the same methods as before were applied to both instruments but with column and column chambers equilibrated to ambient temperature (both instruments were located at the same air-conditioned lab, 2 m from each other). As shown in Figure 7A and D, the situation changed under the new conditions. Peaks eluted slightly later with the Vanquish Horizon system compared to the Acquity system as one could expect from a GDV perspective. These results gave evidence to deviating temperature conditions in the two column thermostats or eluent preheating devices when nominally set to 40 °C. The effective average temperature in the column appeared to be higher with the Vanquish system than with the Acquity system, causing earlier elution. The possible ways to go for a compensation and mimic the conditions of the Acquity instrument were 1) to adapt the column temperature setting, 2) to adapt the active preheater temperature setting, or 3) do both.

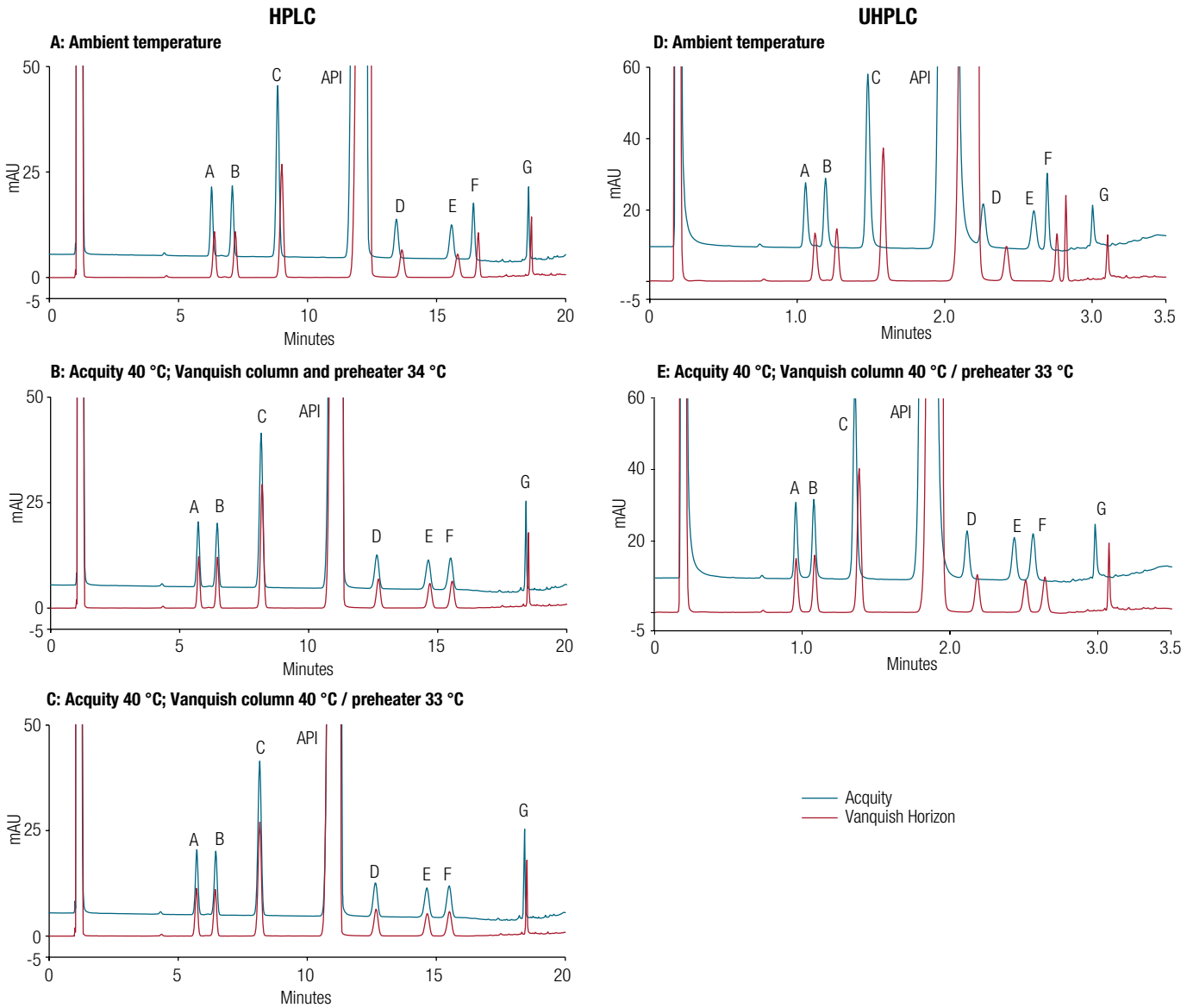

Figure 7. Temperature effects affecting the transfer from Acquity system to Vanquish Horizon system. (A–C) HPLC conditions; (D–E) UHPLC conditions; (A+D) ambient temperature; (B+C+E) Acquity chromatograms with column temperature set to 40 °C but Vanquish Horizon with adjusted temperature settings: (B) Vanquish Horizon column and preheater temperature set to 34 °C; (C) Vanquish Horizon column temperature set to 40 °C and preheater temperature set to 33 °C; (E) Vanquish Horizon column temperature set to 40 °C and preheater temperature set to 36 °C. Peak assignment is according to impurity designation in EP monograph.

For the HPLC method a significant improvement of retention time overlay was achieved as depicted in Figure 7B and C and Figure 8. Reducing the column and preheater temperature at the Vanquish system to 34 °C resulted in t_R deviations of only 0.2% to 0.5% compared to the Acquity system at 40 °C (Figure 7B) and the deviations ranged from 0.3% to 0.5% when the column was kept at 40 °C but only the preheater was set to 33 °C (Figure 7C). In terms of pharmacopeial compliance either technique is applicable to a certain extent. Column temperature adjustments are permitted in a ±10 °C range in the USP guidelines, but only ±5 °C for gradient LC methods in the EP.[11,12] Thus, in the current application one should not go below 35 °C if EP compliance is required. Although the best t_R overlay was obtained at 34 °C, 35 °C will also generate a better t_R fit than keeping the Vanquish Horizon system at 40 °C. However, mobile phase preheating is not addressed in EP or USP monographs and hence the adaption of preheater temperatures is not regulated.

For the UHPLC method no temperature setting was found for the Vanquish Horizon system that generated an overlay of Acquity and Vanquish Horizon data as good as for the HPLC conditions. However, a smaller range of deviations (0.2–3.3%) could be attained by decreasing the active preheater temperature to 36 °C (Figure 7E and Figure 8). In UHPLC methods, as pressure, frictional heating, heat isolation, and heat dissipation play increasing roles, it is much more difficult to emulate the thermostatting of different systems.

Conclusion

- The successful transfer from a Waters Acquity UPLC system to a Thermo Scientific Vanquish Horizon UHPLC system was demonstrated for the EP method for mebendazole impurity analysis. The effort needed to obtain an adequate method transfer highly depends on the requirements of the user.
- Deviations of absolute retention times due to different effective temperatures in the column were compensated by adjustments of column oven temperature or mobile phase preheating temperature.
- Significant savings of 90% eluent consumption and 84% analysis time were obtained by method down-scaling to UHPLC conditions without compromising the chromatographical output.

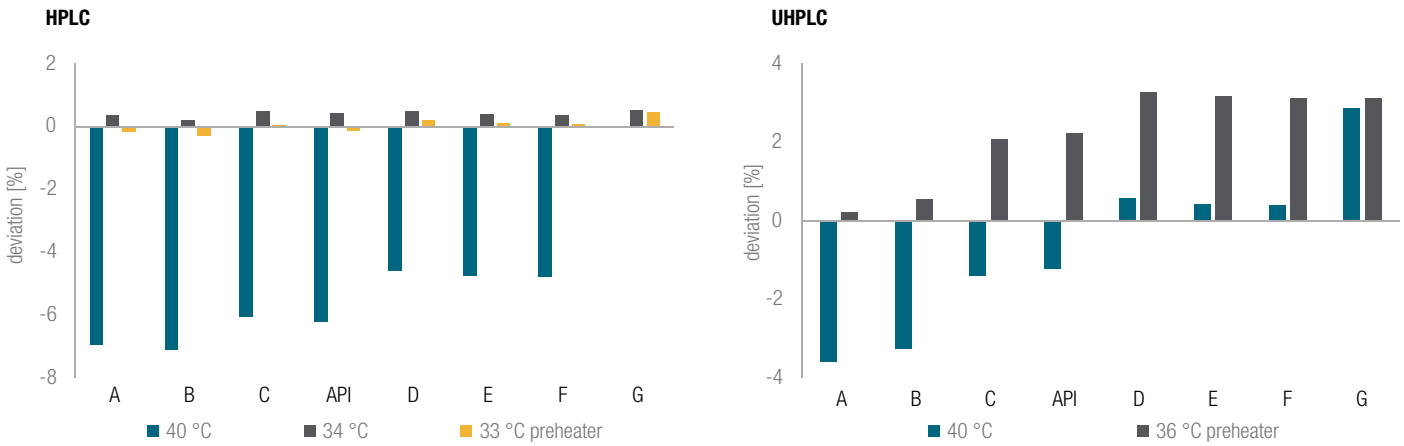

Figure 8. Summary of t_R deviations obtained with the Vanquish Horizon system at different column thermostatting settings with respect to the Acquity system set to 40 °C

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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 Dec 17, 2018).
2. Ermer, J.; Limberger, M.; Lis, K.; Wätzig, H.: The transfer of analytical procedures; *J. Pharm. Biomed. Anal.* **2013,** *85,* 262–276.
3. 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
4. Grübner, M., Paul, C., Steiner F.: Thermo Scientific Application Note 72716: Method transfer of a USP-derived acetaminophen assay from an UltiMate 3000 SD system to a Vanquish Flex UHPLC system, 2018. https://assets.thermofisher.com/TFS-Assets/CMD/Application-Notes/an-72716-lc-method-transfer-acetominophen-ultimate-vanquish-an72716-en.pdf
5. 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
6. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.) Online, 9th edition 2018 (9.5), monograph 0845: Mebendazole.
7. World Health Organization's Model List of Essential Medicines, 20th edition, March 2017, amended August 2017, http://apps.who.int/iris/bitstream/handle/10665/273826/EML-20-eng.pdf?ua=1 (accessed November 30, 2018).
8. The United States Pharmacopeial Convention, United States Pharmacopeia USP41-NF36 1S, Mebendazole monograph, 2018.
9. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.); 7, Allée Kastner CS 30026, F-67081 Strasbourg (France).
10. Thermo Fisher Scientific, HPLC method transfer calculator, https://www.thermofisher.com/us/en/home/global/forms/industrial/lc-method-transfer-calculator.html
11. European Directorate for the Quality of Medicines & HealthCare; European Pharmacopoeia (Ph. Eur.) Online, 9th edition 2018 (9.5), chapter 2.2.46 Chromatographic separation techniques.
12. The United States Pharmacopeial Convention, United States Pharmacopeia USP41-NF36 1S, General chapter <621> Chromatography, 2018.

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