Strategies for the Transfer of Liquid Chromatographic Methods Between Different Instruments

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ABSTRACT

Purpose: To evaluate the effect of different LC instrument parameters on method transfer.

Methods: Two pharmaceutical methods and five different LC instruments were utilized.

Results: Gradient delay volume, column thermostatting, pre-column volume and DAD bandwidth settings should be considered during the transfer of LC methods between different LC instruments.

INTRODUCTION

A challenging task that frequently occurs in all kinds of analytical industries is the transfer of liquid chromatographic (LC) methods from one instrument to another. This is straightforward in case the transfer is between identical instruments. However, the situation becomes more complicated when instruments of different configurations, generations and/or vendors are used. As all LC hardware components to some extent have influence on the chromatographic results, instrumental differences will also affect the analytical outcome of a transferred LC method. Method robustness as well as the degree of instrumentational deviation determine the analytical deviation. Means to counteract these effects depend on the requirements of the operator. For example, if adequate resolution and congruent quantitative results are obtained and sufficing, no effort in adaption is needed. However if in addition retention times need to fit exact specifications, the effort might increase.[1,2]
In our study we investigated several strategies to overcome difficulties in method transfer caused by hardware differences between several instrumental platforms. Quaternary and binary systems were considered. Here our focus was on Thermo Scientific™ Ultimate™ 3000 UHPLC Systems, Thermo Scientific™ Vanquish™ UHPLC Systems, Agilent Infinity 1260 and Waters Acquity. We examined strategies to modify system dwell volumes such as different mobile phase mixers and sample loop sizes as well as the adjustable delay volume of the Thermo Scientific™ Vanquish™ Split Sampler. The effect of pre-column volumes on peak shape was demonstrated for strong solvent injections and the impact of detector settings like the bandwidth was shown.

MATERIALS AND METHODS

Samples

Sample 1: solution of the active pharmaceutical ingredient (API) acetaminophen (1 mg/mL) and its impurities (according to USP[3]) B, C, D, J and 4-aminophenol (10 µg/mL each) in methanol

Sample 2: EP reference standard mebendazole for system suitability[4] (containing API and impurities A, B, C, D, E, F and G according to EP[5]) in dimethylformamide at 1 mg/mL

Experimental

Chromatographic methods are given in Table 1 and were derived from the pharmacopeial monographs: USP monograph for acetaminophen[3] and EP monograph for mebendazole[5]. Instruments used in the current study are summarized in Tables 2 and 3. The acetaminophen assay was applied with quaternary systems, the mebendazole method with binary systems. System control and data analysis was performed with Thermo Scientific™ Chromeleon™ 7.2.9 CDS software.

Table 1. Chromatographic conditions

	Acetaminophen	Mebendazole
Column	Thermo Scientific™ Hypersil GOLD™ C8 column, 4.6x100 mm, 3 µm, 175 Å (p/n 25203-104630)	Thermo Scientific™ Hypersil GOLD™ C18 Selectivity LC column, 4.6x100 mm, 3 µm, 175 Å (p/n 25003-104630)
Eluents	A: 1.7 g/L KH_2PO_4 and 1.8 g/L of Na_2HPO_4 in water B: Methanol	A: 7.5 g/L Ammonium acetate in water B: Acetonitrile
Gradient	min % A % B 0 99 1 3 99 1 7 19 81 7.1 99 1 12 99 1	min % A % B 0 80 20 15 70 30 20 10 90 25 10 90 25.1 80 20 30 80 20
Flow rate	1 mL/min	1.2 mL/min
Column temp.	35 ° C (with eluent preheating)	40° C (with eluent preheating)
Inj. volume	1 µL	5 µL
Detection	230 nm, 10 Hz data collection rate, 0.5 s response time	250 nm, 10 Hz data collection rate, 0.5 s response time / normal filter time (0.2 s)

Table 2. Utilized quaternary systems

	Agilent 1260 Quaternary	UltiMate 3000 SD Quaternary	Vanquish Flex Quaternary
Pump	Quaternary pump (G1311B)	Standard quaternary pump LPG-3400SD (p/n 5040.0031)	Quaternary pump F (p/n VF-P20-A)
Sampler	High Performance Autosampler (G1367E) with thermostat module (G1330B)	Wellplate Autosampler WPS-3000TRS (p/n 5840.0020) with 7 µL eluent preheater (p/n 6722.0540)	Split Sampler FT (p/n VF-A10-A)
Column Compartment	TCC with 6 µL heat exchanger (G1316A)	TCC-3000SD (p/n 5730.0010)	Column Compartment H (p/n VH-C10-A)
Detector	Diode array detector DAD VL (G1315D)	Diode array detector DAD-3000 (p/n 5082.0010)	Diode array detector DAD FG (p/n VF-D11-A)
Flow Cell	standard: 10 mm, 13 µL (G1315-60022)	analytical: 10 mm, 13 µL (p/n 6082.0100)	standard bio: 10 mm, 13 µL (p/n 6083.0540)

Table 3. Utilized binary systems

	Acquity	Vanquish Horizon
Pump	Binary Solvent Manager	Binary Pump H (p/n VH-P10-A)
Sampler	Sample Manager	Split Sampler FT (p/n VF-A10-A)
Sample loop	10 µL	default 25 µL (V=50 µL, p/n 6850.1911)
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)

RESULTS

Gradient delay volume adaption

The gradient delay volume (GDV) of a LC system is defined as the volume between the point of gradient mixing and the column entry. Contributors are pump, sampler and capillary volumes. As the GDV delays the arrival of a particular solvent composition at the column it has a strong impact on elution times. Thus during method transfer GDV adaptions are frequently applied to compensate retention time differences between the sending and receiving LC system.[2] For the transfer of the acetaminophen assay from a Thermo Scientific™ UltiMate™ 3000 SD system to a Thermo Scientific™ Vanquish™ Flex system only a fine-tuning of the GDV was required, which can be accomplished by the adaption of the idle volume of the metering device in the Vanquish autosampler. Figure 1 shows the working principle of that device and Figure 2 the overlaid chromatograms before and after adaption.
If the range of the idle volume (up to 100 µL) is not sufficient for the GDV adjustment the next level is the replacement of the sample loop by a higher volume one. Figure 3 depicts an example for the transfer from an Agilent 1260 system to a Vanquish Flex system by exchanging the default loop (25 µL, V=50 µL) by the 100 µL loop (V=130 µL) and fine tune by the metering device.
However, for GDV differences of major amount a change of the mobile phase mixer should be considered. In Figure 4 that approach is shown for the transfer from an UltiMate 3000 SD instrument to an Agilent 1260 system. Substitution of the 350 µL static mixer by the 750 µL mixer distinctly overcompensated the actual GDV difference. Thus a gradient prestart was applied to match retention times, meaning that the gradient program started at a negative time (-0.27 min) and the injection was conducted at 0 min. All three strategies achieved very good results for all compounds, which eluted during the gradient, without any detrimental effects on the chromatographic performance.
However, the GDV does not affect isocratically eluted peaks like the 4-aminophenol peak in Figures 2-4. Mismatches here might be caused by slight deviations in mobile phase proportioning or column thermostatting.

Figure 1. Vanquish autosampler metering device

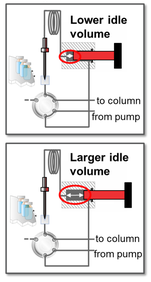


Figure 2. Acetaminophen assay before (A) and after (B) GDV adaption by the idle volume of the metering device in the Vanquish autosampler

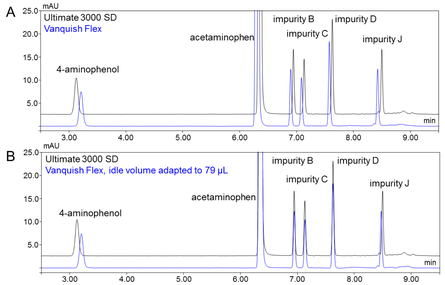


Figure 3. Acetaminophen assay before (A) and after (B) GDV adaption by sample loop and idle volume of the metering device in the Vanquish autosampler

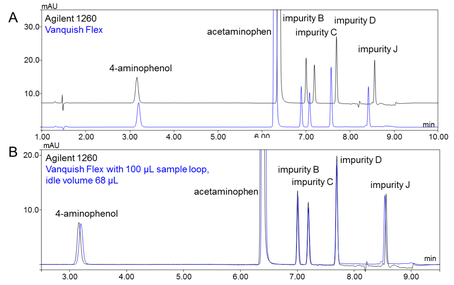


Figure 4. Acetaminophen assay before and after GDV adaption by static mixer exchange (A) and after applying a gradient prestart

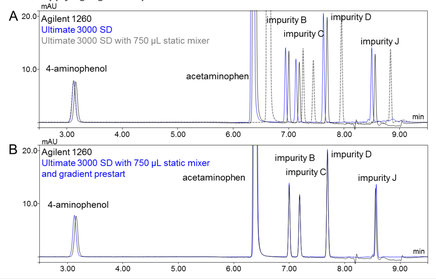
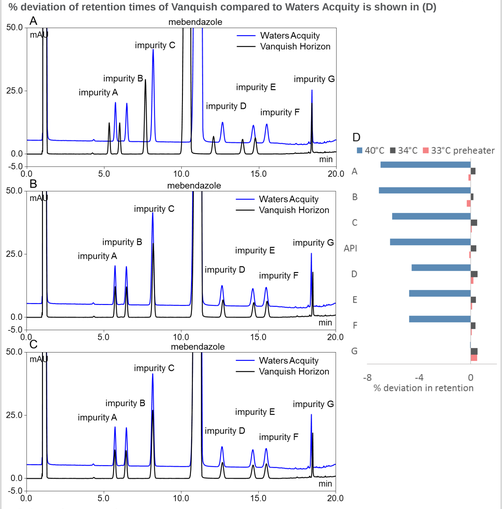


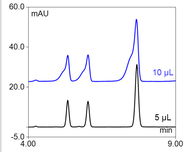
Figure 5. Mebendazole anaylsis with Waters Acquity system set to 40 °C and Vanquish Horizon system set to 40 °C (A), 34 °C(B) and 40 °C with active eluent preheating set to 33 °C; % deviation of retention times of Vanquish compared to Waters Acquity is shown in (D)



Column thermostatting

Very distinct effects of unequal column thermostatting were observed during the transfer of a mebendazole analysis from a Waters Acquity system to a Vanquish Horizon system. Large differences in retention time, which were observed when both column thermostats were nominally set to 40 °C, were eliminated by reducing the temperature to 34 °C at the Vanquish system or by reducing only the temperature of the active solvent preheater to 33 °C (Figure 5), indicating a more efficient column thermostatting of the Vanquish system.

Figure 6. Peak fronting in mebendazole analysis as result of strong sample solvent and high injection volume



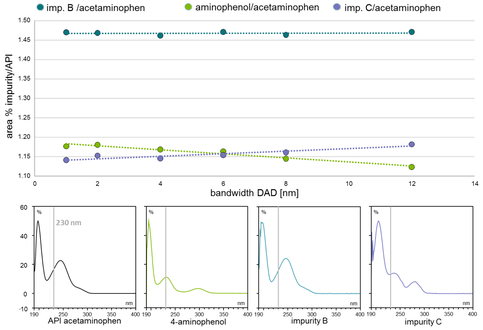
Solvent mismatch effects

Transferring LC methods developed for 4.6 mm i.d. columns to low-volume UHPLC instruments can result in marked peak distortion if large injection volumes are used in combination with sample solvents of strong elution power as seen in Figure 6 for the Vanquish Horizon system. As UHPLC systems are optimized in terms of extra-column volumes the mixing of sample plug and mobile phase prior to the column entry is limited. Thus fronting and peak splitting can result. Strategies to overcome such mismatch effects are reduction of injection volumes, use of sample solvents with less elution power or the integration of additional system volume between sampler and column.

Detector settings.

As the peak area ratio is frequently used for relative quantification, we compared the outcome of several bandwidth settings of a Vanquish DAD. Figure 7 shows very similar UV spectra for the API and impurity B. Thus their area ratio is constant. In contrast, the spectra of 4-aminophenol and impurity C deviate from the API. As a consequence the relative quantification is affected by the bandwidth setting (in different directions for both compounds). Thus, detector settings should be carefully evaluated during method transfer, particularly when different vendor instruments are used.

Figure 7. Peak area ratios of impurities related to the API in dependence of detector bandwidth



CONCLUSIONS.

- Several strategies for GDV difference compensation during method transfer were evaluated and resulted in straightforward retention time matches of sending and receiving LC system.
- Column thermostatting is a critical parameter in method transfer and solvent mismatches of sample and mobile phase should be avoided.
- The bandwidth settings of diode array detectors impact the relative peak ratios of compounds with different UV spectra and should be carefully evaluated during method transfer.

REFERENCES (if necessary)

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TRADEMARKS/LICENSING

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