Instrumental Parameters that affect Method Transfer in UHPLC Separations

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ABSTRACT
The transfer of liquid chromatography methods from one system to another is an extremely frequent practice in analytical laboratories. In principle, a transferred method is identical to the chromatogram for the same sample and different instruments, even though, in practice, some degree of difference is expected and tolerated. The transfer of a UHPLC method may be affected by substantial challenges due to the intrinsic design differences of the systems. Several instrumental parameters have an influence on method transfer. These parameters are: gradient formation, gradient delay volume, extent of sample zone mixing with the surrounding eluent, eluent pre-heating, column oven settings and design and retention factor. The column's radial mixing behavior and flow cell characteristics affect the success of the transfer.

It is possible to predict the impact of hardware deviations on the transfer of UHPLC methods from one system to another. In this work, examples are given of instrumental aspects that can be optimized to reduce such effects. Successful method transfer may in some cases require the change of parameters that are prescribed in the method, which is acceptable in many non-regulated environments. This scenario is demonstrated by the use of advanced instrumental settings.

INTRODUCTION
There are many contributing elements and parameters to be considered when methods are transferred between different HPLC systems as is shown in Figure 1. Gradient methods, the type of gradient generation (LPG vs. HPG) will affect gradient proportioning and flow characteristics. Both the pump with its valves and the autosampler contribute to gradient delay volume (GDV) and gradient shape. System fluidic and fluidic connections define extra column dispersion contribution that affects both the final peak shapes and the pre-injection. Figure 2 shows the effect of increasing volume sample on elution to prior to the column. An often overlooked contribution is related to the type of column still air and forced air. (Integral in Figure 3 shows the influence of increasing sample zone with eluent prior to the column. The large volume contribution is related to the type of column, still air and forced air, and pre-injection. Finally, detector flow cell design and electronic settings are relevant. In this work, we review effects of pre-column mixing, autosampler GDV contribution, column thermostating and eluent pre-heating and we show technical solutions.

Figure 1. Overview on instrumental parameters relevant for method transfer.

MATERIALS AND METHODS
UHPLC Instrumentation
The Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system consisted of a Thermo Scientific™ Vanquish™ Quaternary Pump F, a Thermo Scientific™ Vanquish™ Split Sampler FT, a Thermo Scientific™ Vanquish™ Column Compartment H with active pre-heater, and a Thermo Scientific™ Vanquish™ Diode Array Detector H, equipped with a Standard Flow Cell. The Thermo Scientific™ Donnes™ UHPLC 3000 Bicompatible Rapid Separation (BioRS) UHPLC system consisted of a Thermo Scientific™ Donnes™ UHPLC 3000 BioRS Pump, a Thermo Scientific™ Donnes™ UHPLC 3000 BioRS Column Compartment, a Thermo Scientific™ Donnes™ UHPLC 3000 BioRS Column, a Thermo Scientific™ Donnes™ UHPLC 3000 BioRS Detector (DAD) equipped with a 2.5 µL semi micro flow cell. The Thermo Scientific™ UltiMate™ 3000 SD Standard Chromatography system consisted of a Thermo Scientific™ Donnes™ UltiMate™ UPLC-3000D Standard Quadrant Pump, a Vanquish Flex Detector settings and flow cell design. The UltiMate™ 3000SD Standard Thermostatted Column Compartment and a UltiMate™ 3000 Diode Array Detector (DAD) equipped with a 5 µL semi-analytical flow cell.

Method for Pre-Column Mixing Experiments
The goal of the experiment was to measure the influence of the pre-column systems on peak shapes with sample solvents of different elution strengths as a function of the injection volume and rate to mitigate peak distortion. The experiment was conducted on an UltiMate 3000 SD system and a Vanquish Flex system to show the aspect of method transfer. For details on pre-column fluidics with the Vanquish Flex system, please see Figure 4. The UltiMate 3000 system is configured with a 1,42, 1,44, 1,46, 1,48, 1,50, 1,52 retention factor k'.

Chromatographic Methods for Experiment on Column Thermostatting
The goal of this experiment was to study the influence of the thermostatting mode and the temperature settings of the active pre-heater in an application with strong dependence of selectivity on column temperature. The method transfer was conducted from an UltiMate 3000 BioRS system to a Vanquish Flex Quaternary system.

Column: Thermo Scientific™ Acclaim™ RSLC PA2, Polar Advantage II, 2.2 µm, 21 x 150 mm
Eluent: 35% 20 mM phosphate buffer pH 7.65/ methanol, v/v (isocratic dial-a-shot)
Flow rate: 0.55 mL/min, resulting in 760 bar back pressure
Sample: Uracil, dimethylphthalate, methyleneanil, methyleneanil in mobile phase (1 µL injected)
Temperature: 40 °C, 1.00 m/s (forced air) or 0 (still air) on Vanquish Flex system, active pre-heater temperature in “Results”
Detection: UV at 254 nm

RESULTS
Effect of Pre-Column Mixing
In an ideal HPLC world, the sample dissolved in the mobile phase at the beginning of the gradient. In practice the sample solvent often deviates, but stronger eluting sample solvents can lead to severe peak deformation, in particular when larger sample volumes are injected. Figure 2 shows the effect of increasing sample volume under different solvent conditions measured on a Vanquish Flex column. In Figure 3 is shown the effect of increasing sample zone with eluent prior to the column. The large volume contribution is related to the type of column, still air and forced air, and pre-injection. Finally, detector flow cell design and electronic settings are relevant. In this work, we review effects of pre-column mixing, autosampler GDV contribution, column thermostating and eluent pre-heating and we show technical solutions.

Figure 2. Effect of strong sample solvents as a function of injection volume.

Figure 3. Effect of pre-column fluidics on early eluting peaks at 5 µL injection volume as consequence of varying extent of sample zone mixing.

Conclusions
Fine-tuning of Autosampler GDV Contribution with Mixing Device
The change of the gradient mixer in the pump is a way to vary the GDV of an instrument. However, this can only stepwise change GDV and does not allow exact seamless tuning of this parameter, which can be required if an exact match retention times is demanded, e.g. if peaks in certain applications elute close to each other. The settable volume of the metering device in the Vanquish autosampler defines the position of the metering piston at the point of sample injection. As illustrated on top of Figure 4, this parameter can be used to fine-tune the system GDV in addition to change of gradient mixers. Figure 4 also demonstrates how the exact match of retention times between different systems can be achieved, when the idle volume is varied from the default value (in this example with a reduction by 10 µL).

Figure 4. Adjusting autosampler GDV contribution with metering device.

Effect of Column Thermostatting and Eluent Pre-heating
The separation of preserves on a polar embedded reversed phase with methanol containing mobile phase (Figure 5A) exhibits a strong dependence of selectivity on column temperature. Figure 5B shows the van’t Hoff plot indicating that even a change of elution order between methyl paranol and dimethyl phthalate occurs at 48 °C. Consequently, the resolution of this peak pair is impacted by changes of the effective column temperature as a consequence of different thermostatting modes. The independent setting of the active eluent pre-heater temperature provides a measure to compensate for a heat dissipation in still air mode (D = 0). The resulting change of the effective average column temperature and the effective radial temperature gradient has a pronounced influence on this application. Figure 5C shows that the eluent temperature of the Vanquish Flex system in still-air mode must be lowered by 6 °C to mimic the retention of the UltiMate TCC-3000D Standard Thermostatted Column Compartment which operates in forced air mode. The Vanquish Flex system provides improved efficiency in still air mode. Figure 5D shows how this efficiency can be further improved by lowering the eluent inlet temperature. Due to compensation of radial temperature gradients the still air efficiency is实际行动 improved by an increased temperature difference. The temperature improvement under successful reproduction of retention times leads to 22% improved resolution in a critical peak pair.

Figure 6. Method transfer from UltiMate 3000 Standard Quadrant system to a Vanquish Flex system. The latter was operated in still air mode for best efficiency under elution heating, but with the active pre-heater temperature set equal to column compartment temperature. While the efficiency was improved, the retention and peak shape was not considered. Figure 6B shows the effect of increasing active pre-heater temperature and the latter is characterized by less pre-column mixing as a consequence of optimization for smallest extra column dispersion. Additional pre-column volume i.e. larger bore tubing and mixing devices can be used as fix to mimic the original system.

Figure 7. As Figure 5, but lower active pre-heater temperature to compensate the fractional heating.

CONCLUSIONS
In the case of strongly eluting sample solvents with the surrounding mobile phase can lead to peak deformation when methods are transferred to systems that are optimized for lowest peak dispersion. A possible fix is to build in large bore tubing or even small mixing devices between injector and column.

A variable set-point of an autosampler metering device is a feature that allows fine tuning of the gradient delay volume. This feature can help for exactly matching retention times in gradient methods run on different systems, if this is needed.

In UHPLC methods that generate fractional heating in the column, the transfer from a thermostat with forced air to another one with still air principle will lead to different effective temperatures inside the column. In applications where the efficiency depends on temperature, this can have dramatic effects on peak resolution. If the mobile phase incoming temperature can be separately controlled with an active pre-heater, it is a possible fix to set the pre-heater to a lower temperature than the column compartment to compensate for the temperature increase by fractional heating.

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