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 should deliver identical chromatograms 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 and gradient delay volume, extent of sample zone mixing with the surrounding eluent, eluent pre-heating, column oven settings and design and frictional heat dissipation. Also detector settings 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. In gradient methods, the type of gradient generation (LPG vs. HPG) will affect gradient proportioning and flow characteristics. Both the pump with its mixers and the autosampler contribute to gradient delay volume (GDV) and gradient shape. System tubing and fluidic connections define extra column dispersion contribution that affects both the final peak shapes and the pre-mixing of the injected sample zone with eluent prior to the column. An often overlooked contribution is related to the type of column thermostatting (in particular in the presence of frictional heating) and the eluent pre-heater specifics. Finally, detector flow cell design and electronic settings are relevant. In this work, we review effects of pre-column mixing, autosampler GDV-contribution, column thermosttating and eluent pre-heating and we show technical solutions.

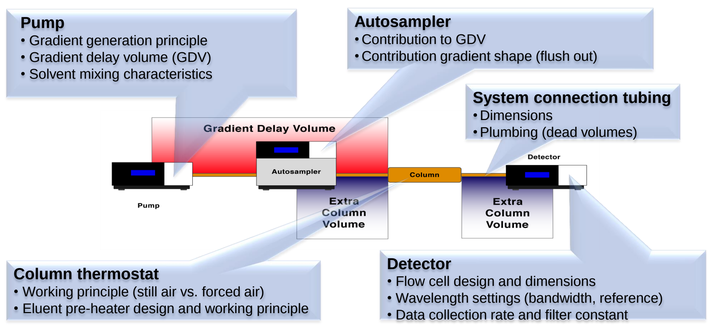

Figure 1. Overview on instrumental parameters relevant for method transfer.

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 HL equipped with a Standard Flow Cell.

The Thermo Scientific™ Dionex ™ UltiMate™ 3000 Biocompatible Rapid Separation (BioRS) Quaternary UHPLC system consisted of a Thermo Scientific™ Dionex ™ UltiMate™ LPG-3400RS Biocompatible Quaternary Pump, a Thermo Scientific™ Dionex ™ UltiMate™ WPS-3000TBRS Biocompatible Autosampler, a Thermo Scientific™ Dionex ™ UltiMate™ TCC-3000RS Column Compartment with 1 µL passive pre-column heater, and a Thermo Scientific™ Dionex ™ UltiMate™ 3000 Diode Array Detector (DAD) equipped with a 2.5 µL semi-micro flow cell.

The Thermo Scientific™ UltiMate™ 3000 SD Quaternary system consisted of a Thermo Scientific™ Dionex ™ UltiMate™ LPG-3400SD Standard Quaternary Pump, a WPS-3000TRS Autosampler, a Thermo Scientific™ Dionex ™ UltiMate™ TCC-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 this experiment was to measure the influence of the pre-column system fluidics on peak shapes with sample solvents of different elution strengths as a function of the injection volume and ways 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, see Figure 3.

Column: Hypersil Gold, 1.9 µm, 2.1 × 50 mm

Eluent: Acetonitrile/water (1/1, isocratic), Flow rate: 0.5 mL/min

Sample: Uracil, 4-Nitroaniline, Methyl Benzoate, Phenetole, o-Xylene solved in Acetonitrile/water (1/1), Acetonitrile or Methanol; 0.5-20 µL injected

Temperature: 30 °C, eluent pre-conditioning with active pre-heater on Vanquish Flex system and no pre-heater on UltiMate 3000 SD system

Detection: UV at 254 nm

Method for Autosampler-GDV-Tuning Experiments

The goal of this experiment was to demonstrate the effect of the Vanquish autosampler metering device idle volume setting for fine-tuning of the autosampler GDV-contribution. Method transfer was conducted from an UltiMate 3000 BioRS system to a Vanquish Flex Quaternary system.

Column: Accucore XL C18, 4 µm, 3 ×100

Gradient: From 20% B to 95% B in 4 minutes (A – H_20, 0.1% H_3PO_4;
B - Acetonitrile, 0.1 % H_3PO_4 , Flow rate: 1.125 mL/min

Sample: Pain killer mix (see peak assignments in Figure 4)

Temperature: 30°C, eluent pre-conditioning with active pre-heater on Vanquish Flex system or passive pre-heater on UltiMate 3000 SD system

Detection: UV at 215 nm

Chromatographic Methods for Experiment on Column Thermostatting

The goal of this experiment was to study the influence of the thermostatting mode and the temperature setting of the active pre-heater in an application with strong dependency of selectivity on column temperature. The method transfer was conducted from an UltiMate 3000 Bio RS system to a Vanquish Flex Quaternary system.

Column: Thermo Scientific™ Acclaim™ RSLC PA2, Polar Advantage II, 2.2 µm, 2.1 x 150 mm

Eluent: 35% 20 mM phosphate buffer pH 7/ 65% methanol, v/v (isocratic dial-a-mix)

Flow rate: 0.55 mL/min, resulting in 760 bar back pressure

Sample: Uracil, dimethylphthalate, methylparaben, methylbenzoate in mobile phase (1 µL injected)

Temperature: 40 °C, fan speed 5 (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 is 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 system. Runs in A are with sample dissolved in mobile phase, B and C with sample in pure organic solvents. The adverse effect of peak splitting is more pronounced with early eluting peaks, as the related substances are not as easily retained to fall back into the eluent. Peak deformation increases with injection volume and starts at 5 µL for an injection from both solvents but is more severe with MeOH than with ACN. Figure 3 shows the transfer from an UltiMate 3000 Standard system to the Vanquish Flex system, where the first shows relatively good mixing of the sample zone with the surrounding eluent and the latter is characterized by less pre-column mixing as a consequence of optimization for smallest extra column dispersion. Additional pre-column volume like larger bore tubing and mixing devices can be used as fix to mimic the original system.

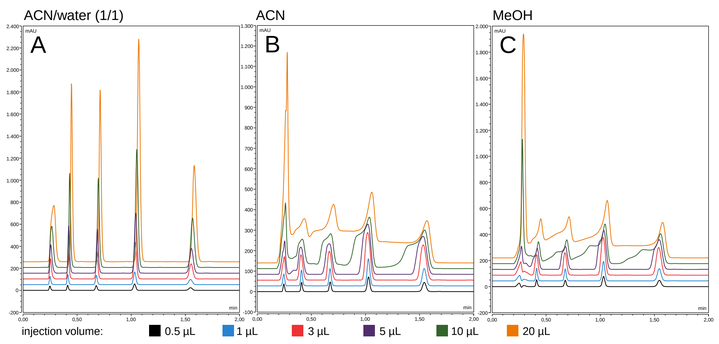

Figure 2. Effect of strong sample solvents as a function of injection volume.

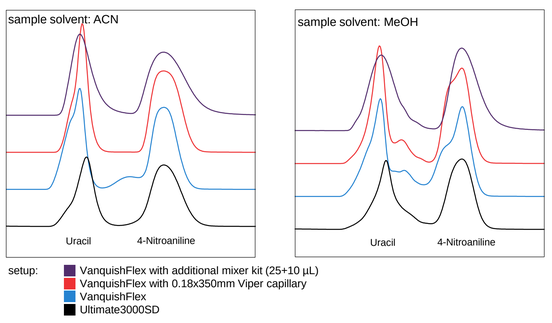

Figure 3. Effect of pre-column fluidics on early eluting peaks at 5 µL injection volume as consequence of varying extent of sample zone mixing.

Fine-tuning of Autosampler GDV-Contribution with Metering 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 idle 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 two different systems can be achieved, when the idle volume is varied from the default value (in this example with a reduction by 10 µL).

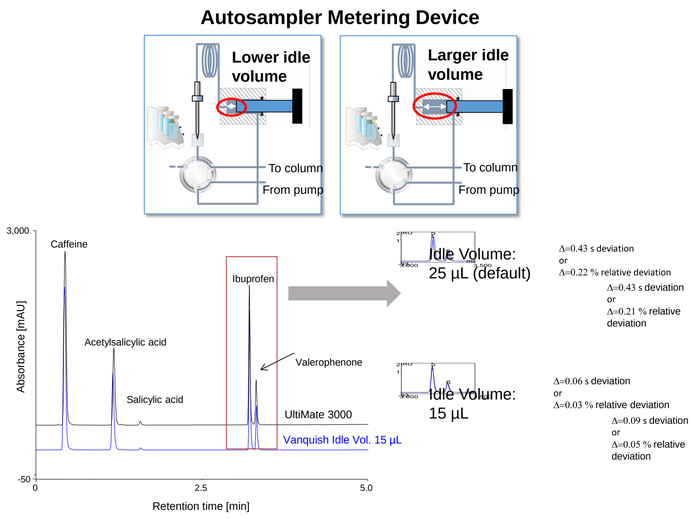

Figure 4. Adjusting autosampler GDV-contribution with metering device.

Effect of Column Thermostatting and Eluent Pre-heating

The separation of preservatives 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 paraben 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 less heat dissipation in still air mode (fan speed = 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 inlet temperature of the Vanquish Flex system in still air mode must be lowered by 8 °C to mimic the retention of the UltiMate TCC-3000SD 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 additionally improved (10% plate count increase instead of 8%). The overall efficiency improvement under successful reproduction of retention times leads to 22% improvement in critical pair resolution (see Figure 5D).

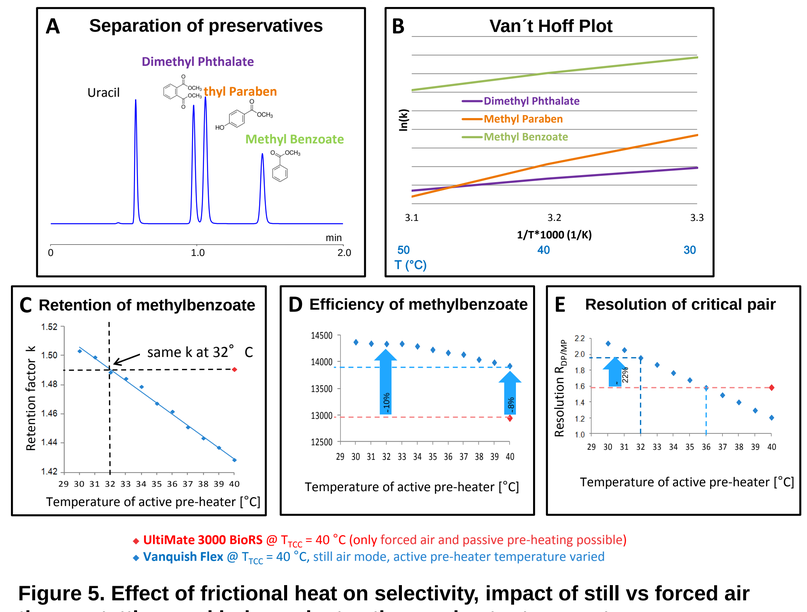

Figure 5. Effect of frictional heat on selectivity, impact of still vs forced air thermostatting, and independent active pre-heater temperature.

Figure 6 shows the method transfer from an UltiMate 3000 Standard Quanternary system to a Vanquish Flex system. The latter was operated in still air mode for best efficiency under frictional heating, but with the active pre-heater temperature set equal to column compartment temperature. While the efficiency was improved, the retention and selectivity dropped due to higher effective column temperature (less distribution of frictional heat). Figure 7 shows how this problem can be fixed by lowering the active eluent pre-heater temperature. The fortunate result is a method transfer with good reproduction on retention times and also improved critical pair.

Figure 6. Method transfer from UltiMate 3000 to Vanquish with T(Pre-heater) = T (Compartment).

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

CONCLUSIONS

- Incomplete mixing 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 system 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 frictional heating in the column, the transfer from a thermostat with forced-air principle to another one with still-air principle will lead to different effective temperatures inside the column. In applications where selectivity 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 frictional heating .

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