A More Flexible Column Thermostatting Technique in LC Method Transfer

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Key Words

Vanquish UHPLC System, Column Compartment, Mobile Phase Preheating, Thermal Mismatch, Frictional Heat, Forced Air, Still Air

Executive Summary

Method transfer is more than adjusting method parameters. When it comes to frictional heat due to viscous heating, the column thermostatting principle plays an important role regarding the actual separation temperature inside the LC column. The Thermo Scientific™ Vanquish™ UHPLC system provides two complementary column thermostatting principles, the still air and forced air mode, and incorporates active mobile phase pre-heating for perfect temperature control. The actual separation temperature strongly depends on the temperature of the mobile phase that enters the LC column. During method transfer, the thermostatting technique of an original LC system can be recreated with the Vanquish UHPLC system.

Introduction

Transferring an HPLC method from one system to another can lead to different results although the gradient delay volumes were adjusted and the same column was used. In some cases, the principle of column and mobile phase thermostatting plays an important role regarding HPLC method transferability. If the mobile phase is not pre-conditioned to column compartment temperature, the temperature within a column can either be similar to the temperature of the mobile phase that enters the column — or thermal mismatch occurs within a column if heat exchange between the column wall and mobile phase is being forced.

UHPLC columns packed with sub-2-micron particles implicate the use of ultra high pressures. At ultra high pressures, frictional heating occurs. Depending on the column thermostatting principle, the temperature increase by frictional heating can easily exceed the value of 15 °C. If frictional heat is dissipated from a column, heat exchange between mobile phase and column wall is being forced which also leads to a thermal mismatch.

However, frictional heat that stays within the UHPLC column can lead to selectivity changes. By lowering either the temperature of the mobile phase or the column compartment down, frictional heat can be outbalanced. Hence, mobile phase preheating must be considered for any LC method transfer and frictional heating effects for UHPLC conditions.
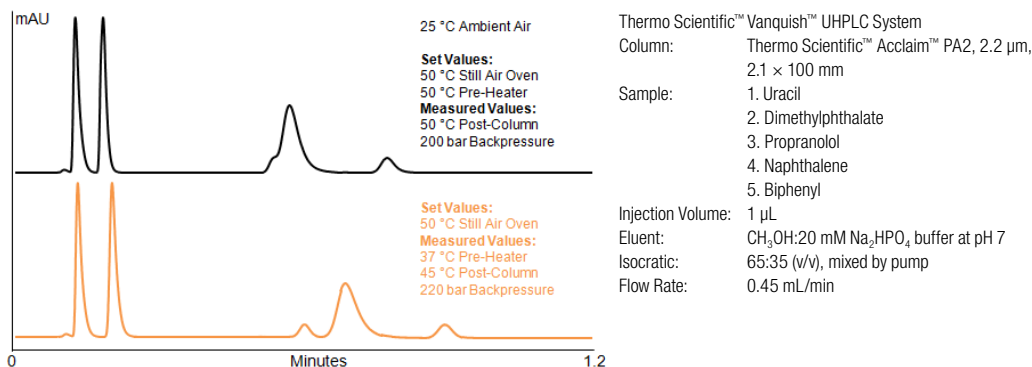
This White Paper describes effects of thermal mismatch on chromatographic results by either insufficient mobile phase pre-condition or frictional heat dissipation from the column. It also describes the effect of frictional heat on analyte retention and separation efficiency. This paper provides hints for proper LC method transfer with regard to column thermostatting options and mobile phase pre-conditioning.

What Impact has Thermal Mismatch on Analyte Retention?

The actual separation temperature inside an LC column is not only defined by the column compartment temperature. The temperature of the mobile phase that enters the column can influence the separation temperature as well. If the mobile phase is not pre-conditioned to oven temperature, a thermal mismatch occurs inside the column. Heat is exchanged between the column wall and center which leads to a radial temperature gradient. Flow rate and column thermostatting principle determine the slope of this radial temperature gradient.
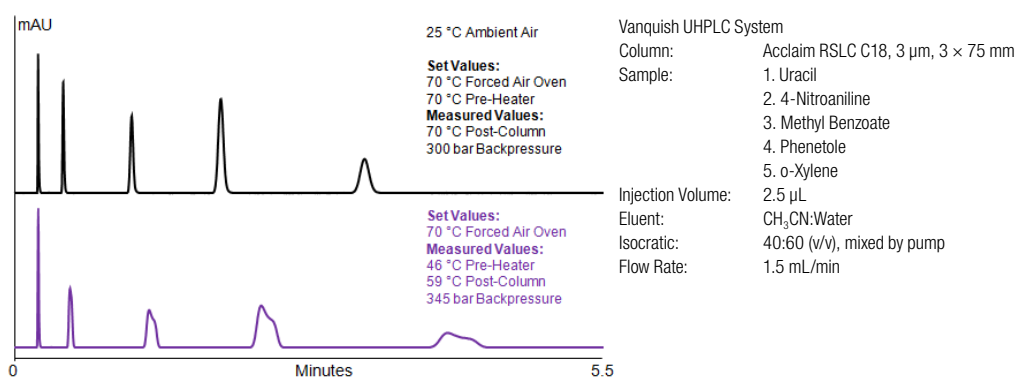
With a still air thermostat, the heat exchange through the column wall is minor. Thus a pronounced radial temperature gradient is prevented. The average separation temperature within the column stays close to mobile phase temperature, especially at higher linear velocities and absence of frictional heat. If the mobile phase is not pre-heated accurately, retention time shifts and even selectivity changes might occur (Figure 1).

Figure 1. Influence of mobile phase pre-heating with still air column thermostatting.

25 °C Ambient Air

Set Values:
50 °C Still Air Oven
50 °C Pre-Heater
Measured Values:
50 °C Post-Column
200 bar Backpressure

Set Values:
50 °C Still Air Oven
Measured Values:
37 °C Pre-Heater
45 °C Post-Column
220 bar Backpressure

Thermo Scientific™ Vanquish™ UHPLC System
Column: Thermo Scientific™ Acclaim™ PA2, 2.2 µm, 2.1 × 100 mm
Sample:
1. Uracil
2. Dimethylphthalate
3. Propranolol
4. Naphthalene
5. Biphenyl
Injection Volume: 1 µL
Eluent: CH_3OH:20 mM Na_2HPO_4 buffer at pH 7
Isocratic: 65:35 (v/v), mixed by pump
Flow Rate: 0.45 mL/min

With a forced air thermostat, heat exchange through the column wall is quite efficient. A pronounced radial temperature gradient is promoted, even at moderate linear velocities. The induced temperature difference leads to different mobile phase viscosities between column wall and center. Analyte retention depends on temperature and eluent viscosity. With different viscosities, the linear velocity becomes different as well. In regions of higher temperature, the linear velocity is higher and analyte retention is lower. Hence, analyte molecules in the column wall region move at a different speed than those in the column center. This results in peak broadening, peak distortion, or even peak splitting (Figure 2). The slope of the radial temperature gradient becomes smaller with a decrease of the linear velocity or a decrease of the column diameter.
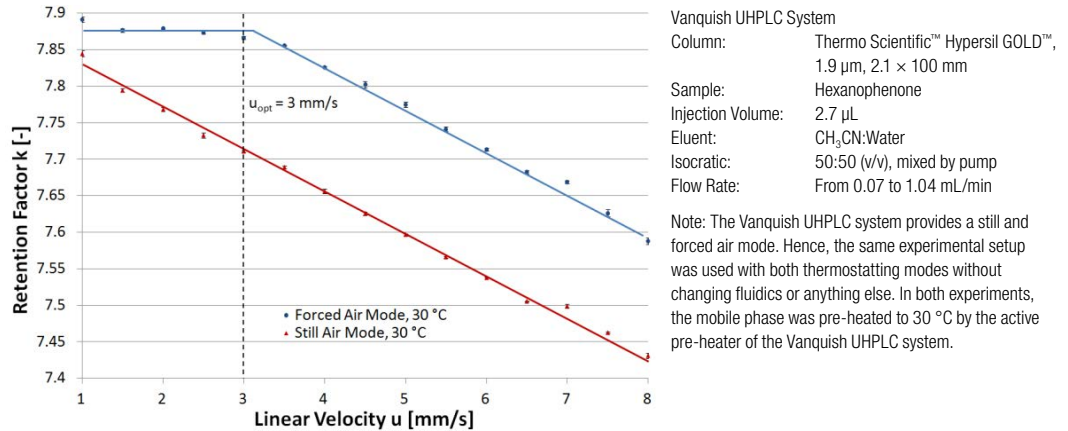
Figure 2. Influence of mobile phase pre-heating with forced air column.

25 °C Ambient Air

Set Values:
70 °C Forced Air Oven
70 °C Pre-Heater
Measured Values:
70 °C Post-Column
300 bar Backpressure

Set Values:
70 °C Forced Air Oven
Measured Values:
46 °C Pre-Heater
59 °C Post-Column
345 bar Backpressure

Vanquish UHPLC System
Column: Acclaim RSLC C18, 3 µm, 3 × 75 mm
Sample:
1. Uracil
2. 4-Nitroaniline
3. Methyl Benzoate
4. Phenetole
5. o-Xylene
Injection Volume: 2.5 µL
Eluent: CH_3CN:Water
Isocratic: 40:60 (v/v), mixed by pump
Flow Rate: 1.5 mL/min

When Should Frictional Heating be Taken into Account?

In general, small particle stationary phases generate heat by viscous friction of the mobile phase, especially under ultra high column backpressures. A column thermostat that enables efficient removal of this frictional heat avoids a pronounced temperature increase inside the LC column. Although the average separation temperature might increase a bit, it would not be that much higher than the set column compartment temperature. If the heat exchange between column thermostat and column wall is suppressed, the frictional heat stays inside the column. Hence, the average separation temperature increases axially from column inlet to column outlet as frictional heat moves with the mobile phase into flow direction.

Figure 3. Influence of frictional heat on retention, depending on thermostatting principle of the column oven.

Vanquish UHPLC System
Column: Thermo Scientific™ Hypersil GOLD™, 1.9 µm, 2.1 × 100 mm
Sample: Hexanophenone
Injection Volume: 2.7 µL
Eluent: CH$_3$CN:Water
Isocratic: 50:50 (v/v), mixed by pump
Flow Rate: From 0.07 to 1.04 mL/min

Note: The Vanquish UHPLC system provides a still and forced air mode. Hence, the same experimental setup was used with both thermostatting modes without changing fluidics or anything else. In both experiments, the mobile phase was pre-heated to 30 °C by the active pre-heater of the Vanquish UHPLC system.

The retention factor of an analyte should not change when the flow rate is increased because, in theory, the retention times of the dead time marker and the analyte change at the same percentage. In reality, more and more frictional heat is produced with increasing flow rate. The heat exchange between column interior and thermostat depends on thermal conduction and convection. With this, a thermostat is only able to dissipate heat from a column until its maximum heat conversion rate is reached. As soon as this thermostat type specific value is exceeded, no further frictional heat is diverted from the column by the thermostat and the average separation temperature inside the LC column rises. For the absolute rise of temperature other factors like the internal diameter of the column, play a role as well.
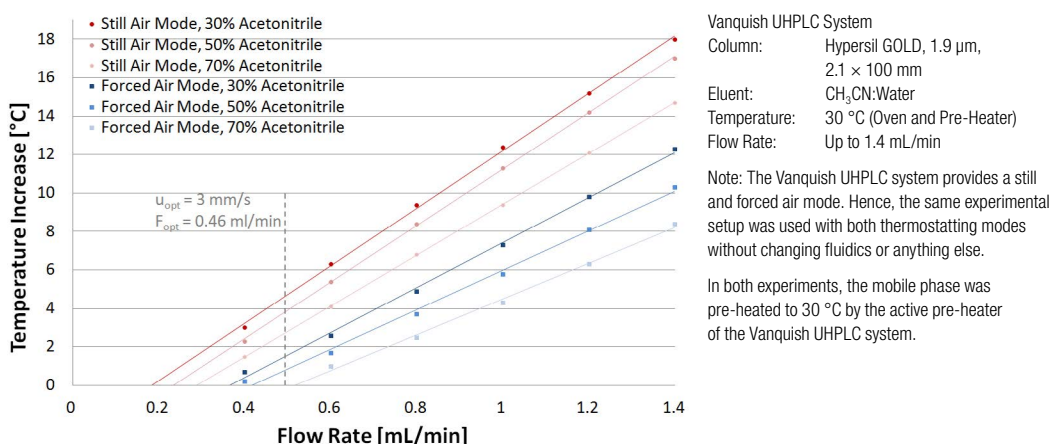
In Figure 3, the retention behavior of a neutral analyte, hexanophenone, was investigated under still or forced air column thermostatting condition. With still air condition, a column is kept in an adiabatic state and frictional heat does not dissipate from the column. This statement has been proved by the constantly decreasing retention factor with the still air mode. Under forced air condition, the retention factor remains constant as long as the column is kept isothermal. This statement was proven in the experiment until a linear velocity of 3.5 mm/s or a flow rate of 0.5 mL/min was reached. Beyond this flow rate, further generated frictional heat remained but did not dissipate from the column anymore and the retention factor began to decrease. This decrease had the same slope which was observed with still air condition. A same slope indicates that an equal amount of frictional heat was produced per step of flow rate increase under both thermostatting modes.

Ideally, a column is operated at its optimum linear velocity (u_{opt}). In this case, u_{opt} is 3 mm/s. This value fits to the region in which the retention factor remained constant with forced air mode while it already decreased with still air mode (Figure 3).

Which Amount of Frictional Heat can be Expected?

To obtain values of frictional heat, a second pre-heater device was connected to the column outlet. Without heating the mobile phase that eluted from the column, its temperature was measured. The mobile phase temperatures at column inlet and outlet were simultaneously recorded by the Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System. The flow rate was increased stepwise and, then, held for ten minutes. No change in the effluent temperature was observed after up to five minutes, depending on the applied flow rate. The axial temperature increase by the frictional heating was plotted against the corresponding flow rate (Figure 4).

Figure 4. Formation of an axial temperature increase according to the applied flow rate.

Vanquish UHPLC System
Column: Hypersil GOLD, 1.9 µm,
             2.1 × 100 mm
Eluent: CH_3CN:Water
Temperature: 30 °C (Oven and Pre-Heater)
Flow Rate: Up to 1.4 mL/min

Note: The Vanquish UHPLC system provides a still and forced air mode. Hence, the same experimental setup was used with both thermostatting modes without changing fluidics or anything else.

In both experiments, the mobile phase was pre-heated to 30 °C by the active pre-heater of the Vanquish UHPLC system.

In literature, mobile phases with high organic content are described to generate much more frictional heat than high aqueous mobile phases. This is caused by the specific heat capacities of acetonitrile and methanol which are much higher than the one of water. This theoretic statement is based on the assumption that the column backpressure remains the same, independent of the organic content of the mobile phase. But at the same flow rate, the column backpressure decreases with increasing organic percentage in the mobile phase. This column backpressure decrease is disproportionately higher than the increase of the specific heat capacity of the mobile phase. Hence, less frictional heat results with an increased organic content in the mobile phase (Figure 4).
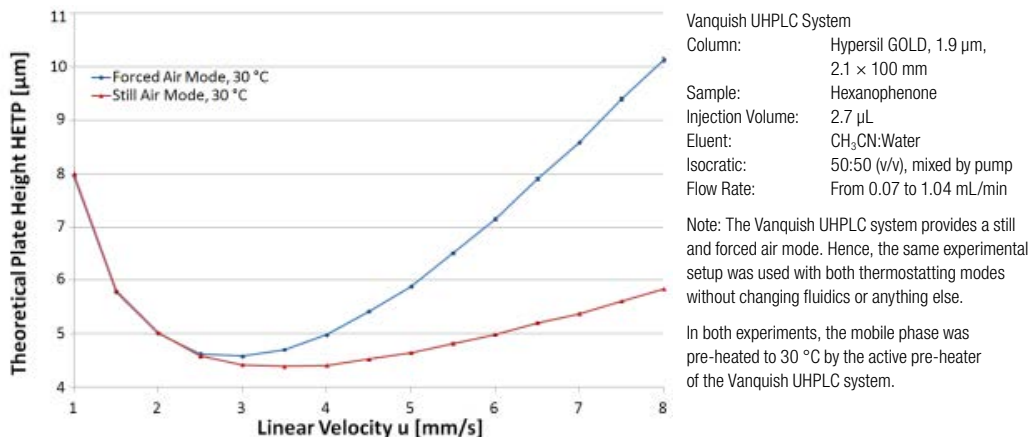
Values of an axial temperature increase were measured for certain isocratic mobile phase compositions under forced and still air conditions. Thereby, the frictional heat formation during mobile phase gradients can be assumed. At the optimum linear velocity (u_{opt}) or flow rate (F_{opt}), an axial temperature increase of 0 up to 2 °C was observed for the forced air mode. When the still air mode was used, the temperature increased axially and between 3 and 5 °C, depending on the mobile phase composition. When running mobile phase gradients at the optimum flow rate, a small temperature fluctuation of 2 °C would occur inside the column. Usually, a chromatographic result is not compromised by that fluctuation.

With a mobile phase containing 50% acetonitrile at a flow rate of 1.4 mL/min, e.g., an axial temperature gradient of 10 °C resulted when the forced air mode was used. With still air mode, the mobile phase that eluted from the column was 17 °C hotter than it was before entering the column. Consequently, 7 °C of frictional heat effect were dissipated through the column wall under forced air condition, thereby forming a radial temperature gradient. Although 7 °C might not sound like too much, the chromatographic result is altered.

How Much is Separation Efficiency Affected?

The optimum linear velocity (u_{opt}) for best separation efficiency is shown by a Van Deemter curve. It also predicts efficiency loss when the actual linear velocity is below or beyond u_{opt}. Small particle stationary phases account for fast analyte diffusion into the stationary phase and back. Theoretically, little efficiency loss should occur at very high linear velocities for superfast analysis with smallest particle stationary phases. However, any adverse effect from increased viscous heating at elevated linear velocities is not considered in this statement.

Figure 5. Van Deemter curves of hexanophenone with either forced air or still air conditions.

Vanquish UHPLC System

Column:	Hypersil GOLD, 1.9 μm, 2.1 × 100 mm
Sample:	Hexanophenone
Injection Volume:	2.7 μL
Eluent:	CH$_3$CN:Water
Isocratic:	50:50 (v/v), mixed by pump
Flow Rate:	From 0.07 to 1.04 mL/min

Note: The Vanquish UHPLC system provides a still and forced air mode. Hence, the same experimental setup was used with both thermostatting modes without changing fluidics or anything else.

In both experiments, the mobile phase was pre-heated to 30 °C by the active pre-heater of the Vanquish UHPLC system.

An efficiency drop is indicated by the slope of a Van Deemter curve. This slope can have a significant contribution from thermal mismatch due to viscous heating at an elevated linear velocity. Figure 5 shows Van Deemter curves of hexanophenone recorded with increasing viscous heating effect by accelerating the linear velocity. At the most elevated linear velocity of 8 mm/s, the column efficiency with forced air mode is by 40% lower than with still air mode. This efficiency loss is caused by the radial heat removal through the column wall when the forced air mode was used. Here, the still air mode is advantageous if the best achievable efficiency at very high analysis speed is wanted. Moreover, the still air mode accounts for a 10% better efficiency and 20% faster analysis under optimum conditions at a linear velocity of 3 mm/s.

When is the Still Air Mode not the Preferred Choice?

With still air mode, frictional heat is not actively removed from the column. The separation temperature inside the column increases axially, depending on the flow rate. Thereby, a retention factor does not stay constant when using different flow rates (Figure 3). Retention factor changes can be critical for certain applications, especially for samples with very polar compounds (Figure 1). For such different compound classes, retention factors often change irregularly to each other which might result in a change of selectivity and resolution. Retention factors, selectivity, and resolution do not change to that extent under forced air conditions, if the efficiency loss due to frictional heat does not compromise the resolution.

Hence, the chromatographic result may change if the frictional heat contribution is not addressed adequately during a method transfer between two UHPLC systems with a different thermostatted column compartment.

Which Considerations are Important Regarding LC Method Transfer?

First of all, mobile phase pre-heating is essential to gather comparable results with each LC method transfer, independent from HPLC or UHPLC conditions. Retention factors, selectivity, and resolution are temperature-dependent parameters which can change when switching between two LC systems providing different column thermostats and when mobile phase pre-conditioning is handled differently.

During UHPLC method transfer, the usage of different column thermostats lead to different average separation temperatures and also to a certain temperature distribution within the UHPLC column as upcoming frictional heat can either be dissipated from the column or is left inside. In general, the average separation temperature within the column increases differently for forced or still air column thermostats. Similar separation temperatures can be achieved even if the amount of frictional heat is known. The temperature of the mobile phase can be lowered down and the average temperature within the column is adjustable. Hence, the separation temperature is controllable.

The Vanquish Column Compartment H offers complementary column thermostatting modes, still and forced air, at the push of a button. Furthermore, the forced air mode can be stepwise controlled. Active mobile phase pre-heating is also provided. Different temperatures for column compartment and mobile phase are supported. Hence, each and every column thermostat, from still air to forced air, as well as non-, passive, or active mobile phase pre-heating operation can be easily recreated with the Vanquish Column Compartment H.

Conclusions

The actual separation temperature inside a LC column is not to be confused with the set oven temperature. The temperature of the mobile phase can shift the average separation temperature and, thereby, influence the chromatographic result. Furthermore, a thermal mismatch inside the LC column might occur, depending on the column thermostatting technique. Column thermostatting and mobile phase preheating have to be effective, but must not compromise the achievable column efficiency. When it comes to frictional heat due to viscous heating, the column thermostatting technique plays an important role regarding the actual separation temperature, its distribution within the LC column, and the chromatographic result.

During an LC method transfer between two UHPLC systems with a different column thermostat, recreating the proper separation temperature plays an important role. The two column thermostatting conditions, forced and still air mode, or the active mobile phase pre-heating of the Vanquish UHPLC system can be used as valuable tools to recreate other LC column thermostats, to achieve the best possible column performance, or to maintain retention factor and selectivity reproducibility.

References

1. Thermo Scientific White Paper 71499. The Role of Temperature and Column Thermostatting in Liquid Chromatography. 2015.

2. Thermo Scientific Product Spotlight SP71195: Best in UHPLC Column Thermostatting to Fit All Needs. 2014. [Online] http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/bro/bro/chrom/lc/sys/SP-71195-Vanquish-Column-Thermostatting-SP71195-EN.pdf (accessed Jan 27, 2015).

3. Thermo Scientific Poster Note PN71314: Thermostatting in UHPLC: Forced Air Mode, Still Air Mode, and Method Transfer. Germering, Germany, 2014. http://www.thermoscientific.com/content/dam/tfs/ATG/CMD/cmd-documents/sci-res/posters/chrom/lc/sys/PN-71314-ISC2014-UHPLC-Thermostatting-PN71314-EN.pdf (accessed Jan 27, 2015).

4. Dolan, J. Separation Science. HPLC Solutions #53: Temperature and Retention. [Online] https://owl.english.purdue.edu/owl/resource/747/08/ (accessed Jan 27, 2015).

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