

What Efficient Temperature Control Can Teach Us in Liquid Chromatography

Frank Steiner,¹ David Thomas,² Evert-Jan Sneekes,¹ and Michael Heidorn¹

¹Thermo Fisher Scientific, Germering, Germany; ²Thermo Fisher Scientific, Chelmsford, MA, USA



Overview

Purpose: Influences of the mobile phase temperature or frictional heat in combination with the column thermostat type on the chromatographic result shall be evaluated.

Methods: Same method settings compared under different thermostating conditions.

Results: Proper temperature control is more than setting a value for the column thermostat, especially for UHPLC methods or when using elevated temperatures.

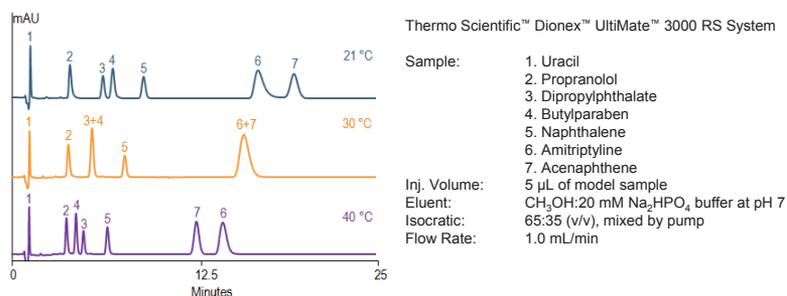
Introduction

Each good chromatographer wants to implement proper temperature control. For this purpose, column thermostats have evolved from simple units just providing a fixed temperature to high-tech devices. Elevated temperatures, well above the boiling point of the mobile phase, become more and more applied. Consequently, mobile phase pre-heating prior to entering the column as well as post-column mobile phase cooling prior to entering the detector becomes mandatory. In addition, viscous heating is a much discussed topic in Ultra High Performance Liquid Chromatography (UHPLC). When pressures increase to values far above 400 bar, friction between column and mobile phase generates high amounts of viscous heat and, thereby, an additional temperature increase inside the column.

In this work, various elements of the entire temperature control (e.g., mobile phase pre-heating or column thermostat type) are evaluated to determine effects of the different thermostating conditions on chromatographic performance. Furthermore, the amount of frictional heat was measured for a typical UHPLC column (1.9 μm , 2.1 x 100 mm). It is discussed how chromatographic results can be maintained or even improved during a method transfer or speed-up.

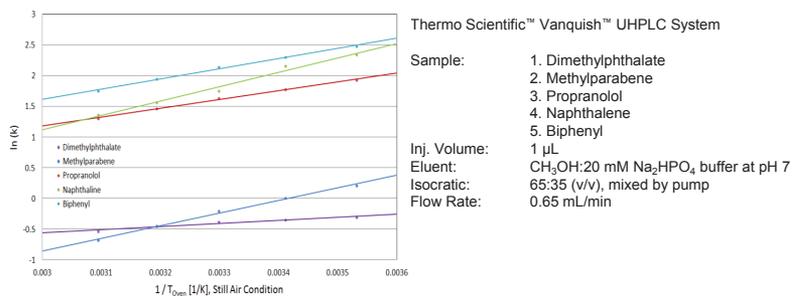
Importance of Temperature Control in LC

FIGURE 1. Influence of the temperature on the chromatographic result.



A rule of thumb for isocratic reversed-phase LC predicts 1—2% retention time decrease for each 1 °C column temperature increase. The percentage decrease in retention time is usually not the same for all compounds of a sample mixture and changes in peak spacing are common. In that case, retention factors of sample compounds change, sample compounds may co-elute, or their selectivities can be inverted by a small variation of the column temperature (Figure 1).

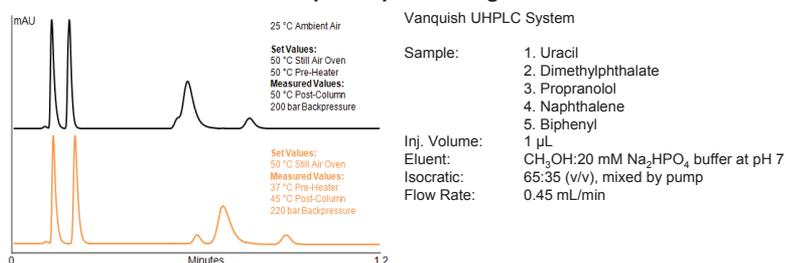
FIGURE 2. Van't Hoff plots for some neutral and polar analyte molecules.



For most molecules and separation conditions, the retention factor k of an analyte changes proportional with temperature (c.f. Van't Hoff equation). In Van't Hoff plots, a $\ln(k)$ value is plotted against the reciprocal value of the corresponding separation temperature (Figure 2). An intersection point of two Van't Hoff curves indicate a change in selectivity for the two corresponding analytes at the given temperature.

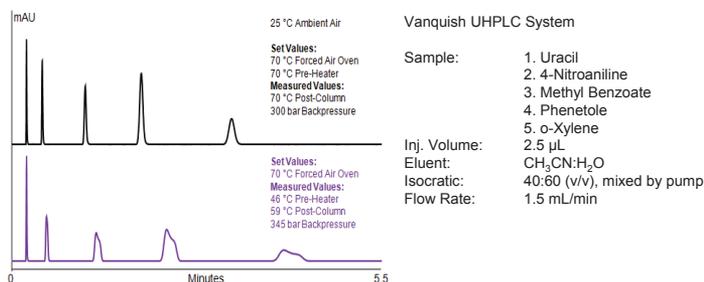
Mobile Phase Pre-Heating

FIGURE 3. Influence of mobile phase pre-heating with a still air thermostat.



With a still air thermostat, retention time shifts and selectivity changes might occur at elevated temperatures if the mobile phase is not pre-heated accurately (figure 3).

FIGURE 4. Influence of mobile phase pre-heating with a forced air thermostat.

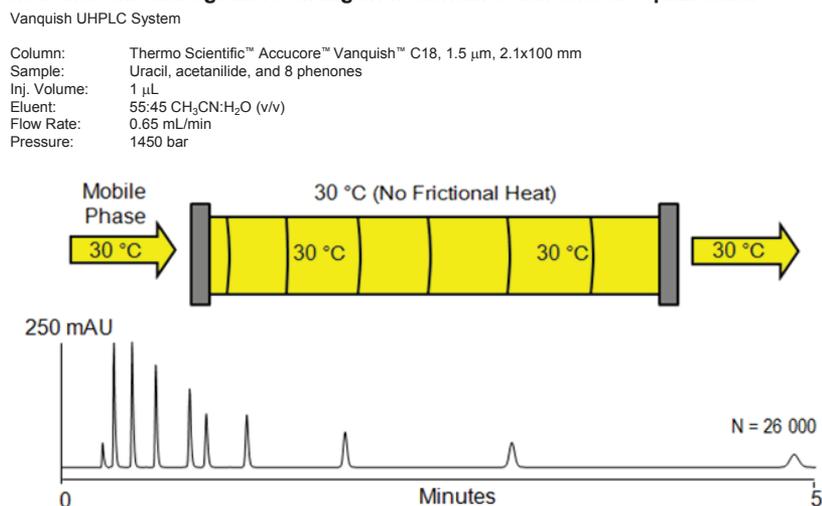


With a forced air thermostat, peak broadening or even peak distortion might occur at elevated temperatures if the mobile phase is not pre-heated accurately (Figure 4).

Post-Column Mobile Phase Cooling

When running separations at elevated temperatures, boiling of organic mobile phases might appear or vapor bubbles might outgas in the detector. During separation, both is suppressed by a high system pressure. Frictional heating at ultra high pressures might lead to similar situations. Increased baseline noise, ghost peaks or even complete light absorption can appear in the detector flow cell. A post-column mobile phase cooler should be used. It also maintains sufficient backpressure for proper detector operation.

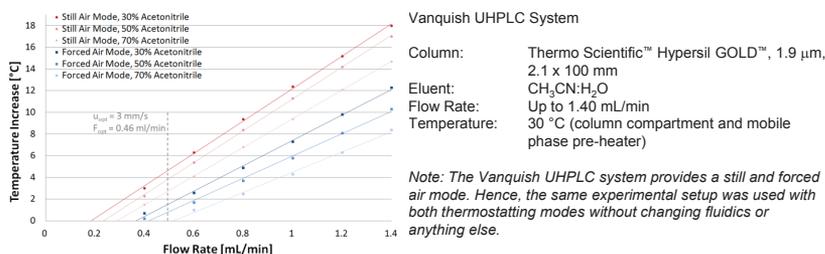
FIGURE 5a. Temperature distribution inside an LC column without frictional heat and chromatogram resulting with thermal conditions at equilibrium.



Note: To mimic a "no frictional heat" situation, the mobile phase was actively pre-heated to an inlet temperature of 23 °C. Caused by frictional heat, the mobile phase had a temperature of 39 °C at the column outlet. Hence, an average separation temperature of approximately 30 °C can be assumed, theoretically leading to a similar result as with a thermal equilibration state at 30 °C.

Temperature Gradients by Frictional Heat

FIGURE 6. Axial temperature increase in correlation to the flow rate.



In Figure 6, the axial temperature increase within a typical UHPLC column is plotted against the flow rate. It is shown for different isocratic mobile phase compositions.

TABLE 1. Axial temperature increase for a 1.9 μm, 2.1 x 100 mm column with a mobile phase containing 50% acetonitrile related to backpressure and flow rate.

50% Acetonitrile Flow Rate [mL/min]	Still Air Mode		Forced Air Mode	
	System Backpressure [bar]	Axial Temperature Increase [°C]	System Backpressure [bar]	Axial Temperature Increase [°C]
0.2	173	0.0	177	0.0
0.4	343	2.3	356	0.2
0.6	506	5.4	527	1.7
0.8	659	8.4	685	3.7
1.0	809	11.3	830	5.8
1.2	958	14.2	975	8.1
1.4	1079	17.0	1099	10.3

With a mobile phase containing 50% acetonitrile at a flow rate of 1.04 mL/min or linear velocity of 8 mm/s, e.g., an axial temperature gradient of 6 °C results with forced air mode and 11.5 °C with still air mode. Consequently, 5.5 °C of frictional heat are dissipated through the column wall under forced air condition, thereby forming a radial temperature gradient. As shown in Figure 7, the chromatographic results are enormously altered.

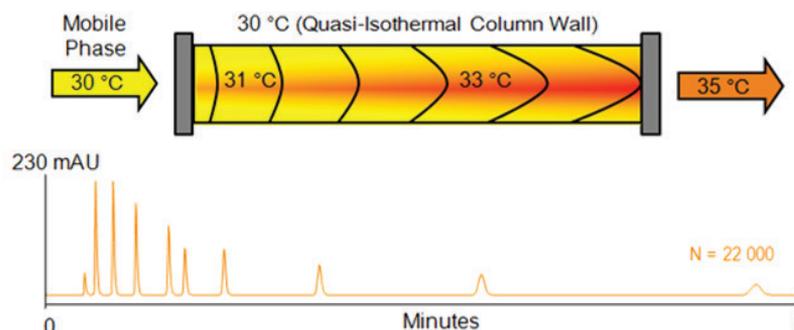
Frictional Heat Effects on Chromatography

As depicted in Figure 6, the column thermostating mode has a big influence on the axial temperature increase as soon as frictional heat is dissipated. Then, radial temperature gradients are formed within the column. Figures 5 a-c demonstrate theoretic temperature distributions inside an LC column under typical, different column thermostating conditions. The effect on the separation efficiency (theoretical number of plates, N) as well as analyte retention is shown by authentic chromatograms.

FIGURE 5b. Influence of frictional heat under quasi-isothermal conditions (forced air mode) on temperature distribution and resulting chromatogram.

Thermo Scientific Vanquish UHPLC System

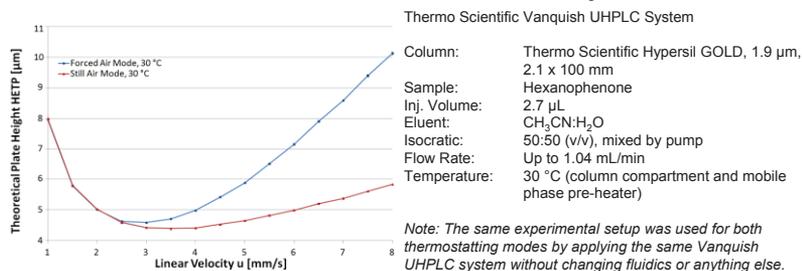
Column: Accucore Vanquish C18, 1.5 μm, 2.1x100 mm
 Sample: Uracil, acetanilide, and 8 phenones
 Inj. Volume: 1 μL
 Eluent: 55:45 CH₃CN:H₂O (v/v)
 Flow Rate: 0.65 mL/min
 Pressure: 1430 bar



Note: For a quasi-isothermal situation, the forced air mode with the highest settable fan speed of the Vanquish UHPLC system was applied. The Vanquish UHPLC system allows to set seven different fan speeds for method transfer reasons.

The Effect of Frictional Heat on Efficiency

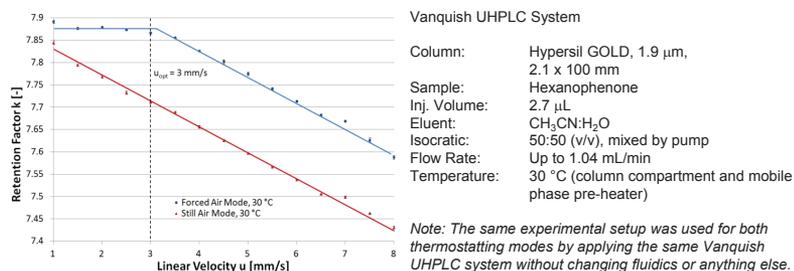
FIGURE 7. Influence of frictional heat on column efficiency.



An efficiency drop is indicated by the slope of a Van Deemter curve. Figure 7 shows Van Deemter curves recorded with increasing viscous heating effect by accelerating the linear velocity. The Vanquish UHPLC system provides a still air and a forced air mode. Except for those two complementary thermostating modes, identical conditions were used to acquire each Van Deemter curve. At the linear velocity of 8 mm/s, the column efficiency with forced air mode was by 40% lower than with still air mode.

The Effect of Frictional Heat on Retention

FIGURE 8. Influence of frictional heat on retention factor.

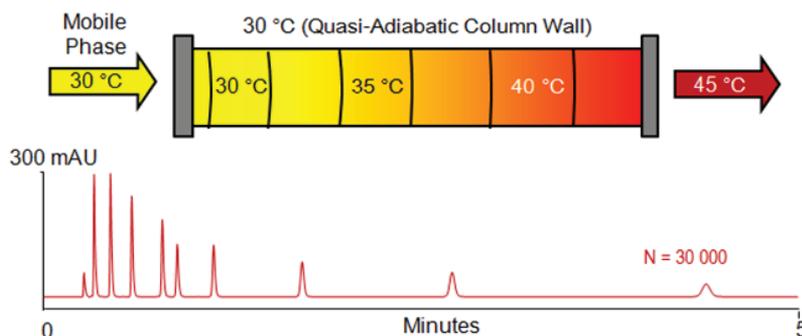


In figure 8, retention factors were investigated with increasing viscous heating by accelerating the linear velocity. With still air mode, the retention factor decreased constantly. With forced air mode, the retention factor remained constant until a linear velocity of 3.5 mm/s or a flow rate of 0.5 mL/min was reached. Beyond this flow rate, the retention factor began to decrease. The same slope for the retention factor decrease indicates that an equal amount of frictional heat was produced per step of flow rate increase under both thermostating modes.

FIGURE 5 c. Influence of frictional heat under quasi-adiabatic conditions (still air mode) on temperature distribution and resulting chromatogram.

Vanquish UHPLC System

Column: Accucore Vanquish C18, 1.5 μm , 2.1x100 mm
 Sample: Uracil, acetanilide, and 8 phenones
 Inj. Volume: 1 μL
 Eluent: 55:45 $\text{CH}_3\text{CN}:\text{H}_2\text{O}$ (v/v)
 Flow Rate: 0.65 mL/min
 Pressure: 1385 bar



Note: Quasi-adiabatic column thermostating can be supposed when still air condition is used. The Vanquish UHPLC system provides a still air mode. For a fast column heat-up even with this still air mode, Thermo Scientific™ Dionex™ Chromatography Data System automatically uses forced air during the temperature equilibration phase, hence, until the set temperature is reached. Afterwards, the fan of the column thermostat is turned off for a certain period of time. The separation is performed under still air condition.

Temperature Control in UHPLC

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 8). Retention factor changes can be critical for certain applications, especially for samples with very polar compounds. For such compound classes, retention factors often change irregular to each other which might result in a change of selectivity and resolution (Figure 1).

Hence, chromatographic results may change if the frictional heat contribution is not addressed adequately during a method transfer from HPLC to UHPLC, between two UHPLC systems with a different column oven, or when a method speed-up is applied especially in UHPLC. 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 chromatographic resolution.

Temperature Settings in Method Transfer

If a method is transferred from HPLC to UHPLC conditions, the flow rate is adjusted but the actual linear velocity is kept constant. Ideally, u_{opt} is used in the HPLC method and maintained for the UHPLC method. In this case, the use of the forced air mode does not significantly compromise the separation efficiency (Figure 7). Retention factors and selectivity are retained while the analysis is generally speeded-up and the resolution is often improved. The still air mode can also be used but retention control might become an issue (Figure 8).

During a method speed-up, when the flow rate is accelerated beyond F_{opt} , the frictional heat contribution to the separation temperature increases. Retention factors cannot be kept constant anymore (Figure 8), even when using the forced air mode. Furthermore, a loss of separation efficiency would occur with the forced air mode. Here, the still air mode is the better choice as the separation efficiency is not affected. To maintain the retention factors and selectivity with the still air mode, frictional heating has to be taken into account. For this purpose, either the mobile phase or the column compartment temperature must be lowered down by half the nominal value of the axial temperature increase resulting from frictional heating (Figure 5a, see comment in the note).

Conclusion

- In each case, mobile phase pre-heating is essential for proper temperature control in LC and to obtain best chromatographic results.
- To maintain proper detector operation, a post-column mobile phase cooler needs to be used when elevated temperatures are applied.
- At column backpressures above 400 bar, frictional heating effects on retention and column efficiency needs to be addressed.
- Retention factors, selectivity, and resolution do not change if u_{opt} and the forced air mode are applied for a method transfer from HPLC to UHPLC conditions.
- For a method transfer from HPLC to UHPLC conditions where the linear velocity is above u_{opt} or when the linear velocity is increased above u_{opt} during a method speed-up, the still air mode should be used and either the mobile phase or the column compartment temperature must be lowered down by half the nominal value of the axial temperature increase resulting from frictional heating.

References

1. Heidorn, Michael. The Role of Temperature and Column Thermostatting in LC. **2015**. Thermo Scientific White Paper 71499.
2. Heidorn, Michael. A More Flexible Column Thermostatting Technique in LC Method Transfer. **2015**. Thermo Scientific White Paper 71500.

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