Thermostatting in UHPLC: Forced Air Mode, Still Air Mode, and Method Transfer

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Overview

Purpose: Trading easiest method portability against ultimate column efficiency in UHPLC.

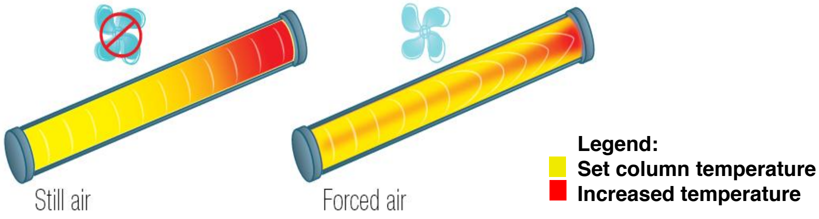
Methods: Different mobile phase compositions, flow rates, column temperatures, and column thermostatting mode.

Results: The still air mode is the solution to counteract any negative impact of frictional heat on column efficiency. The effect of frictional heat on retention can be addressed by lowering the mobile phase temperature.

Introduction

Viscous friction between mobile and stationary phase can generate a considerable amount of frictional heat. Its extent mainly depends on the column backpressure and mobile phase characteristics. Frictional heat can cause radial and axial temperature gradients within a column. Both, thermostatting mode and column diameter influence the formation of a temperature profile in a column (Fig.1). The still air mode prevents from the well-known efficiency loss by frictional heat which occurs with forced air mode. However, the average column temperature increases as the entire frictional heat remains in the column when the still air mode is used. Thereby, changes in retention and, possibly, in selectivity occur. With the forced air mode, frictional heat dissipates from the column and analyte retention should not change significantly.

FIGURE 1. Temperature Profile in a Thermostatted Column with Still (left) or Forced Air (right) Mode.



Van Deemter curves are used to find the optimum linear velocity for the best column efficiency. With small particle UHPLC columns, little efficiency loss should occur even at linear velocities far above the optimum. However, this statement does not account for any adverse effect on the efficiency from increased viscous heating at elevated linear velocities. In this poster, the frictional heat influence on the efficiency is shown by comparing Van Deemter curves which were measured with still or forced air mode.

From the same experimental results, retention factors are plotted against the linear velocity to observe changes in retention with increasing frictional heat. Frictional heat was measured for certain isocratic mobile phase compositions. Thereby, the frictional heat formation during mobile phase gradients can be assumed. Finally, it is shown how column efficiency and retention stability can be combined when a method is transferred from forced to still air conditions.

Methods

Liquid Chromatography System

Thermo Scientific™ Vanquish™ UHPLC system with: Vanquish Binary Pump H, Vanquish Split Sampler HT, Vanquish Column Compartment H, Vanquish Diode Array Detector HL with 10 mm Thermo Scientific™ LightPipe™ flow cell.

Data Analysis

Thermo Scientific™ Dionex™ Chromeleon™ Chromatography Data System software 6.8

Van Deemter Test Conditions

Column: Thermo Scientific™ Hypersil GOLD™ C18 column, 1.9 µm, 2.1 x 100 mm
Sample: Phenones and uracil (5 µg/mL)
Injection : 2.5 µL
Mobile Phase A: Fisher Scientific™ Optima™ LC/MS water
Mobile Phase B: Optima LC/MS acetonitrile
Isocratic : 50% A and 50% B
Flow Rates: 0.07 – 1.04 mL/min
Temperature: 30 °C (oven and preheater)
Oven Mode: see figure
Detection : 240 nm wavelength, 50 Hz data collection rate, 0.1 s response time, 4 nm slit and bandwith

Frictional Heat Measurement

Conditions: If not listed below, same as above
Isocratic: 30% B, 50% B, or 70% B
Flow Rate: 1.30 mL/min
Temperatures: 30 °C or 50 °C (oven and preheater)

Alkyl Phenones Application

Conditions: If not listed below, same as above
Gradient: 0 min 40% B, 0.95 min 100% B,1.15 min 100% B, 1.3 min 40% B, 2 min 40% B
Flow Rate: 1.30 mL/min
Temperature: 50 °C oven, preheater see figure

Results

The Vanquish UHPLC system provides an entirely new and unique combination of active eluent pre-heating with two complementary conditions of column thermostatting, namely the still and forced air mode.

FIGURE 2. Van Deemter Curves of Hexanophenone Recorded with Still or Forced Air Conditions.

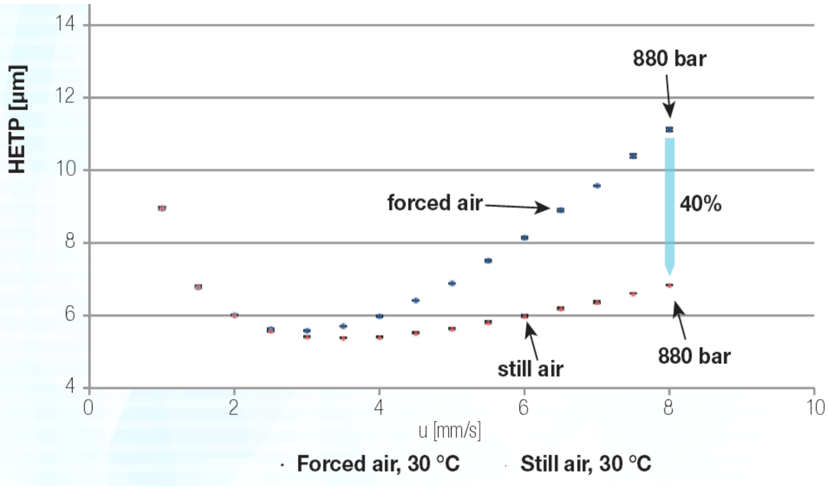
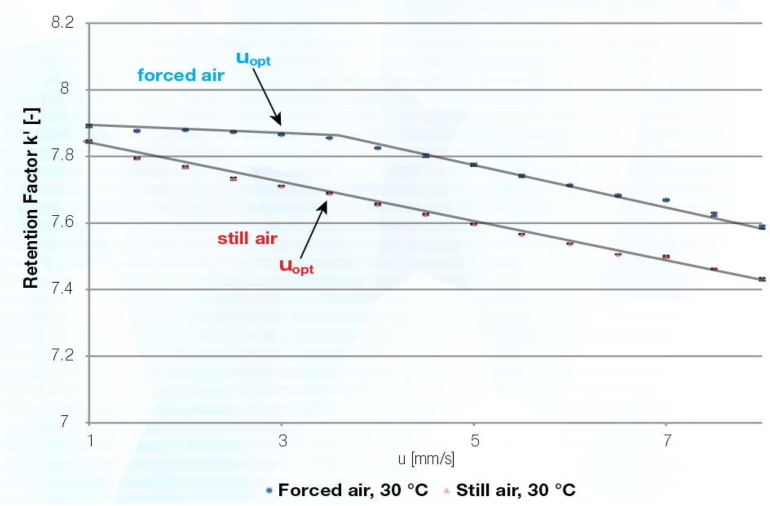


Figure 2 shows two Van Deemter curves for the same analyte recorded under still or forced air condition, and with increasing viscous heating effect as the linear velocity is accelerated. At the most elevated speed of 8 mm/s, the efficiency is by 40% lower when forced air is used instead of still air. This obvious efficiency loss is caused by the removal of frictional heat at the column wall. Forced air condition leads to radial temperature gradients within the column. If ultimate efficiency at fastest analysis speed is wanted, still air condition is apparently the better choice.

FIGURE 3. Retention Factors of Hexanophenone Recorded with Still or Forced Air Conditions.



Retention factors remain constant up to a linear velocity slightly higher than the optimum when forced air condition is applied (Fig. 3). After this, they decrease with increasing linear velocity. Under still air condition, the retention factor drop already starts with the first step-up of linear velocity. Retention factor changes can be very critical for certain applications as selectivity and resolution are dependent on analyte retention. Retention changes should always be considered when a method is transferred from HPLC to UHPLC, between UHPLC systems which contain different column ovens, or with a method speed-up in UHPLC. With forced air condition, the best achievable retention factor reproducibility is given. As long as efficiency loss does not compromise peak resolution much, forced air condition is very useful for method transfer purposes.

TABLE 1. Frictional Heat Values at 30°C Column Oven and Pre-Heater Temperature.

	Still air mode			Forced air mode		
Water/acetontrile (v%/v%), isocratic	70/30	50/50	30/70	70/30	50/50	30/70
Frictional heat (Kelvin)	16.6	15.6	13.4	10.8	9.2	7.4
System back-pressure (bar)	1118	1026	840	1140	1039	860

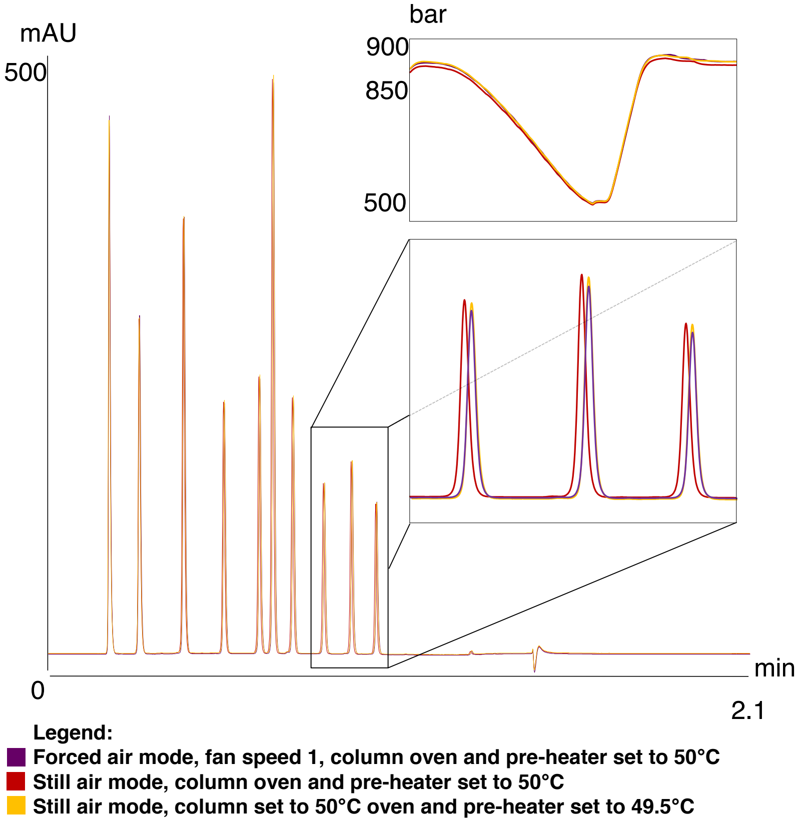
TABLE 2. Frictional Heat Values at 50°C Column Oven and Pre-Heater Temperature.

	Still air mode			Forced air mode		
Water/acetontrile (v%/v%), isocratic	70/30	50/50	30/70	70/30	50/50	30/70
Frictional heat (Kelvin)	12.9	12.2	10.6	8.4	6.8	5.8
System back-pressure (bar)	848	777	666	865	799	686

To obtain values of frictional heat, a second pre-heater device was connected to the column outlet. It was just used to measure the temperature of the mobile phase that eluted from the column without heating it. Mobile phase temperatures at column inlet and outlet were recorded by Chromeleon simultaneously. The difference between both temperatures resulted in the frictional heat values of table 1 and 2. If the mobile phase mixture was changed from high aqueous to high organic content, the frictional heat decreased by 3 Kelvin or even less. When comparing the data points of related test series between still and forced air, approximately 5 Kelvin of frictional heat dissipated from the column under forced air condition.

At the same column backpressure, acetonitrile generates more frictional heat than water. But at the same flow rate, acetonitrile creates less backpressure than water with the same column. If proportions of a mixture are varied, the column backpressure changes when the flow rate is kept constant. Next to this, the frictional heat conversion rate also changes with the composition of the mobile phase. Throughout the experiments, both changes compensated each other concerning the generation of frictional heat. Regardless of the proportions of water and acetonitrile, equal frictional heat values were measured at a constant flow rate. Hence, the formation of the frictional heat profile inside the column would virtually be undisturbed when a mobile phase gradient of water and acetonitrile is run on the Hypersil GOLD C18 column, 1.9 µm, 2.1 x 100 mm.

FIGURE 4. Chromatogram Overlay of the Phenones Application with Different Thermostatting Mode.



The most crucial comparison between still and forced air conditions is done when as little as possible of frictional heat dissipates through the column wall with the forced air mode. The Vanquish UHPLC system allows to change the fan speed of the forced air mode. In Figure 4, the same mobile phase gradient was applied to separate phenones with different thermostatting mode. As a starting point, the forced air mode was used but with 20% of the default fan speed (purple trace). As a next step, it was switched to the still air mode (red trace). A tiny column backpressure drop, a slight decrease in retention times, and 10% increase in column efficiencies were observed. Nevertheless, when the pre-heater was set to a slightly lower temperature with the still air mode (yellow trace), the same retention times and column backpressure were found as with the modified forced air mode. The efficiencies were similar to those of the original still air mode experiment that was run before.

An alternative strategy has recently been published.[1] In that study, a separation was performed with a 2.1 mm I.D. column at 30°C and 100 bar. When the separation was performed at 22°C and 600 bar with still air mode, the same retention factors and selectivity resulted. To obtain the same retention factors and selectivity at 1000 bar, the column oven temperature was lowered to even 14°C.

Conclusion

- At fastest analysis speed, ultimate column efficiency is achieved with the still air mode.
- As long as peak resolution is not compromised much, the forced air mode is the choice for method transfer as retention factors and selectivity do not change.
- For advanced method transfer, the fan speed of the forced air mode can be regulated.
- In particular with the still air mode, the column oven or pre-heater temperature can be adjusted to keep retention factors constant even at fastest analysis speed.
- The Vanquish UHPLC system supports the choice between retention control or efficiency optimization as well as advanced method transfer options.

References

1. Novakova, L.; Veuthey, J. L.; Guillarme, D. *J. Chromatogr. A.* **2011**, 1218, 7971—7981.

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