

Optimizing Selectivity and Efficiency in Critical UHPLC Methods by Separating Eluent Pre-heating from Column Thermostatting

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ABSTRACT

Purpose: UHPLC methods at pressures up to 1500 bar can generate substantial frictional heat and thus impact the effective local temperature in columns. Depending on the column thermostat type such frictional heat will cause either radial or longitudinal temperature gradients, or both. These effects can have significant implications for method optimization, because temperature gradients and effective column temperatures change with column length, flow rate, and mobile phase viscosity. The influence on the chromatogram can be substantial if the selectivity of critical peak pairs depends on temperature.

Methods: We applied a system with pressure capability up to 1500 bar under heat generating conditions in columns of 2.1 mm i.d. packed with particle sizes between 1.5 μm and 2.2 μm . Extensive frictional heat resulted from either methanol containing mobile phases or extended column lengths up to 200 mm. The column thermostat was able to control the degree of heat dissipation by varying the fan speed to alter air circulation as well as setting the active mobile phase pre-heater at a temperature independent of the compartment temperature. The instrument also allowed tracking the eluent temperature after the column by using a second active pre-heater (switched off) as a temperature sensor.

Results: This setup enabled thermal balance studies and effective ways to develop temperature based kinetic and thermodynamic method optimization. While still air thermostatting generated the highest theoretical peak capacities, critical selectivity differences and changes in peak resolution between both thermostatting modes were observed. Setting the pre-heater to a lower temperature than the column compartment to compensate for frictional heating effects in still air mode was found to be a key solution for holistic optimization of both efficiency and selectivity.

INTRODUCTION

Commonly considered best practice in HPLC for effective retention control and best efficiency is to achieve a homogeneous temperature distribution across the entire separation column. UHPLC methods at pressures up to 1000 bar can generate a temperature increase up to 20 °C by frictional heating [1]. This inherently results in temperature gradients in the column, either in radial direction under forced air mode, or axial under still air mode. These differences between isothermal and adiabatic operation are described in literature [2]. However, there are no data reported with different thermostatting modes studied on a commercial column compartment and at pressure levels above 1000 bar. Another aspect not addressed is the setting of active eluent pre-heater temperatures lower than the column compartment temperature. The closer a thermostat operates to adiabatic mode the more frictional heat can impact chromatographic retention. In applications where column temperature affects selectivity, such thermostatting effects can significantly change peak resolution and be a challenge for method transfer between different instruments.

The work described here addresses a comparison of Van Deemter curves in forced and still air mode with additional consideration of how retention factor changes with flow rate and includes systematic recording of the eluent heat-up at pressures up to 1100 bar (Experiment 1). The next experiment takes the conditions to more than 1400 bar column head pressure to study the impact of both modes on efficiency and retention (Experiment 2). The separation of preservatives was used to show the effects of temperature on changes in selectivity, including inverse elution order. The differences between different instruments and the effects of how the independent active pre-heater temperature of the Vanquish systems help to tune the separation were systematically studied (Experiment 3). Lastly the positive impact of fan speed and active pre-heater temperature adjustment on the resolution of an impurity peak in a sample is shown (Experiment 4).

MATERIALS AND METHODS

UHPLC Instrumentation for Experiments 1, 2, and 4

The Thermo Scientific™ Vanquish™ Horizon UHPLC system consisted of a Vanquish Binary Pump H, a Vanquish Split Sampler HT, a Vanquish Column Compartment H with active pre-heater, and a Vanquish Diode Array Detector HL equipped with a Standard Flow Cell (10 mm light path, 2 μL illuminated volume, 0.8 μL dispersion volume).

UHPLC Instrumentation for Experiment 3

The Thermo Scientific™ Vanquish™ Flex Quaternary UHPLC system consisted of a Vanquish Quaternary Pump F, a Vanquish Split Sampler FT, a Vanquish Column Compartment H with active pre-heater, and a Vanquish Diode Array Detector HL equipped with a Standard Flow Cell.

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

Chromatographic Methods for Experiment 1

The goal of Experiment 1 was to record Van Deemter curves and the corresponding retention factor dependency on the linear velocity, as well as temperature increase in columns by frictional heating under still air and forced air conditions. See Figure 1 for technical details and how the mobile phase temperature behind the column was measured.

Column:	Thermo Scientific™ Hypersil™ GOLD, 1.9 μm , 2.1 x 100 mm
Mobile Phase:	50% water / 50% acetonitrile, v/v (isocratic dial-a-mix)
Sample:	Hexanophenone in mobile phase (1 μL injected)
Temperature:	30 °C, fan speed 5 (forced air) or fan speed 0 (still air)
Detection:	UV at 240 nm

Chromatographic Methods for Experiment 2

The goal of Experiment 2 was to study the influence of the thermostating mode on the efficiency and retention of alkylphenones under pressures close to the maximum of the Vanquish Horizon system.

Column: Thermo Scientific™ Accucore™ Vanquish C18, 1.5 μm , 2.1 x 100 mm
Mobile phase: 45% water / 55% acetonitrile, v/v (isocratic dial-a-mix)
Flow rate: 0.65 mL/min
Sample: Uracil, acetanilide, and 8 phenones in mobile phase (1 μL injected)
Temperature: 30 °C, fan speed 5 (forced air) or fan speed 0 (still air)
Detection: UV at 240 nm

Chromatographic Methods for Experiment 3

The goal of Experiment 3 was to study the influence of the thermostating mode and the temperature setting of the active pre-heater in an application with strong dependency of selectivity and resolution on column temperature. The experiment was conducted on an UltiMate 3000 Bio RS and a Vanquish Flex system to also address the aspect of method transfer.

Column: Thermo Scientific™ Acclaim™ RSLC PA2, Polar Advantage II, 2.2 μm , 2.1 x 150 mm
Mobile phase: 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, active pre heater temperature in "Results"
Detection: UV at 254 nm

Chromatographic Methods for Experiment 4

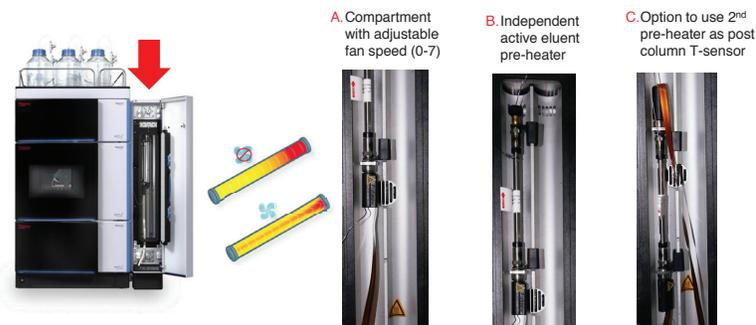
The goal of Experiment 4 was to study the influence of column compartment fan speed and temperature setting of the active pre-heater in an application with a difficult to resolve impurity peak. The sample was obtained from a customer.

Column chain: 2x Thermo Scientific Hypersil GOLD VANQUISH, 1.9 μm , 2.1 x 100 mm coupled in series
Binary gradient: From 5% to 30% B in 4 min, A: 0.1% formic acid in water, B: acetonitrile
Flow rate: 0.8 mL/min
Sample: Sample from customer containing gallic acid, caffeic acid, and salicylic acid (1 μL injected)
Temperature: 40 °C, see Figure 8 for fan speed and active pre-heater settings
Detection: UV at 300 nm

Data Analysis

Thermo Scientific™ Chromeleon™ Chromatography Data System (CDS) software version 7.2.

Figure 1. The Vanquish UHPLC systems feature a vertical column compartment optimized for the use of 2 columns up to length of 30 cm. Air circulation can be adjusted with the 7 different fan speeds or fan switched off (A). The active pre-heater with internal temperature sensor can be set to values different from the compartment (B) and can also be used as post-column temperature sensor to measure heat up of eluents (C).



RESULTS

Experiment 1: Efficiency, Retention, and Column Temperature under Frictional Heating in Still and Forced Air

Isocratic alkylphenone separations have been used on a 1.9 μm fully porous RP column at different flow rates and under both still air and forced air thermostating conditions. Van Deemter curves and retention factor plots were calculated from these data and are shown in Figure 2 for hexanophenone (latest eluting peak). Additional experiments with a second pre-heater as temperature sensor behind the column have been recorded to measure the heat-up under both thermostating modes. For temperature recording experiments the specified maximum column pressure of 1000 bar was exceeded.

The Van Deemter experiments show significantly different curves between both modes with 40% reduction in height equivalent of a theoretical plate (HEPT) at the highest speed of analysis and a curve minimum at 15% higher speed, indicating the efficiency advantage of the still air mode. Figure 2 also shows that in neither mode the retention factor is constant over the entire flow range. Here the advantage of the forced air mode to allow for a constant retention close to the curve minimum (u_{opt}) becomes apparent. Over the entire range the retention factor drops 6% in still air mode and less than 4% in forced air mode.

Table 1 shows heat up rates in both modes as function of the flow rate F. While the data in Figure 2 range from F = 0.1 mL/min ($u = 0.7$ mm/s) to F = 1.1 mL/min ($u = 8.0$ mm/s), to account for column integrity staying below 900 bar, the heat up was studied up to 1.4 mL/min flow rate. In still air mode a heat up of 2 – 3 °C per 0.2 mL/min flow rate change was observed from the lowest flow rate up. In forced air mode this heat-up occurs only above 0.6 mL/min (or 500 bar) in line with the retention factor development observed. Maximum heat up in still air mode was 17 °C. The column head pressure evolved respectively in the two different modes, reflecting the viscosity change with temperature

Figure 2. Van Deemter curves and corresponding retention factor curves for still air and forced air thermostating in isocratic runs at pressures up to 900 bar (Experiment 1).

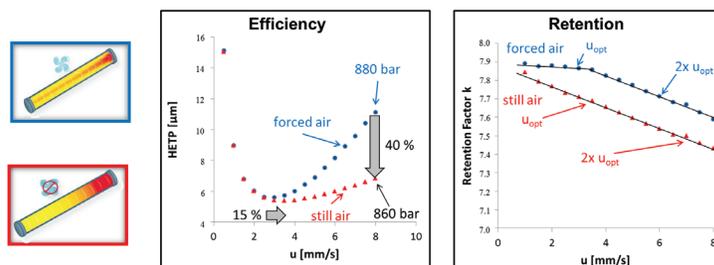


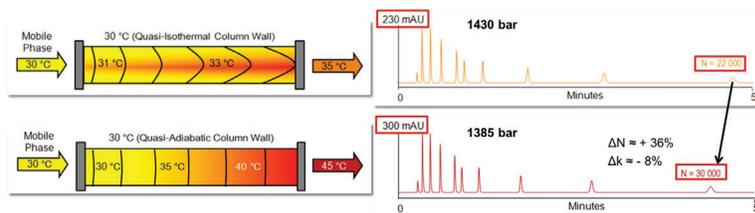
Table 1. Column head pressure and heat up rate under two different thermostating modes (Experiment 1).

Flow rate [mL/min]	Δp (still air) [bar]	ΔT (still air) [°C]	Δp (forced air) [bar]	ΔT (forced air) [°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

Experiment 2: Efficiency and Retention above 1400 bar in Still and Forced Air Mode

The Accucore Vanquish column allows experiments close to the pressure maximum of the Vanquish Horizon instrument. Figure 3 illustrates schematically the temperature distribution and the resulting distortion of band profiles under radial temperature gradients. The chromatograms from both modes are shown at adapted y-axes indicating a signal increase of 30% due to significant efficiency increase in still air mode (36% for plate number N). The retention of octanophenone drops by 8% due to higher average column temperature in still air mode (lower viscosity leads to 45 bar lower pressure).

Figure 3. Schematic illustration of heat generation and dissipation in both modes and alkylphenone chromatograms run on Accucore Vanquish 1.5 μm column under extreme pressure conditions (Experiment 2).

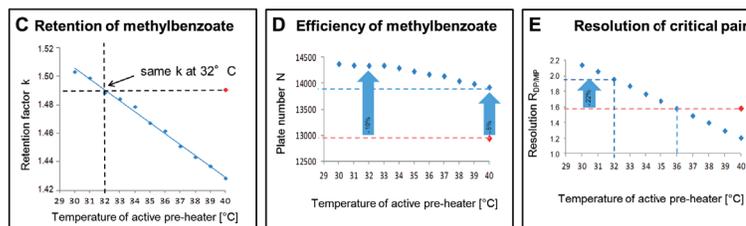
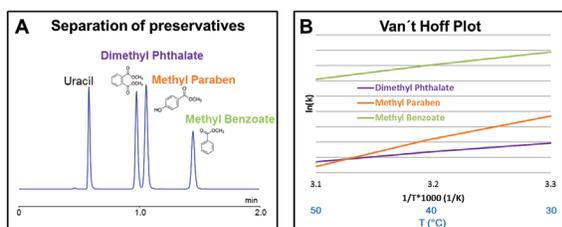


Experiment 3: Selectivity and Resolution Changes in Method Transfer and Counter Measures by Compensating Heat-up with Lower Active Pre-heater Temperatures

The separation of preservatives on a polar embedded reversed phase with methanol containing mobile phase (Figure 4A) exhibits a strong dependence of selectivity on column temperature. Figure 4B 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 thermostating modes. The independent setting of the active eluent pre-heater temperature provides a measure to compensate for less heat dissipation in still air mode. The resulting change of the effective average column temperature and the effective radial temperature gradient has a pronounced influence on this application. Figure 4C shows that the eluent inlet temperature of the Vanquish system in still air mode must be lowered by 8 °C to mimic the retention of the UltiMate 3000 TCC which operates in forced air mode. Obviously the incentive to run Vanquish in still air mode was the improvement of efficiency. Figure 4D shows how this efficiency can even be further improved by lowering the eluent inlet temperature. Due to compensation of radial temperature gradients the still air efficiency is additionally improved (10% plat 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 4D).

These positive effects of separating the active pre-heater temperature from the compartment temperature can be concluded from the chromatograms in Figures 5 and 6. Figure 5 shows the method transfer from UltiMate 3000 to Vanquish operated in still air mode, but with active pre-heater temperature equal to compartment temperature. While the efficiency was improved, the retention and selectivity dropped due to higher effective column temperature (less distribution of frictional heat). This has a dramatic influence on the critical pair resolution. Figure 6 shows how this problem is fixed by lowering the active eluent pre-heater temperature. The result is a method transfer with good reproduction on retention times and also improved critical pair resolution from 1.58 to 1.93.

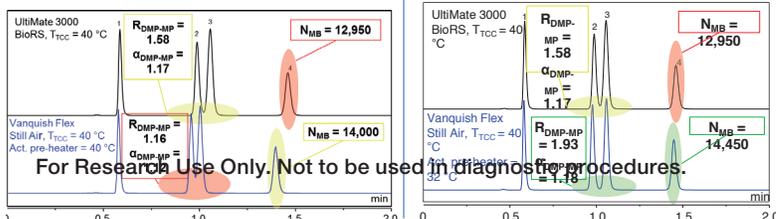
Figure 4. Experimental series demonstrating the effect of frictional heat on selectivity, the impact of still and forced air thermostating, and lowering of active pre-heater temperature to compensate low heat removal in still air thermostats. A) shows the separation under optimized conditions, B) the selectivity change with temperature and C-E) the dependence of retention, efficiency and critical pair resolution on $T_{pre-heater}$



- UltiMate 3000 BioRS @ $T_{TCC} = 40$ °C (only forced air and passive pre-heating possible)
- Vanquish Flex @ $T_{TCC} = 40$ °C, still air mode, active pre-heater temperature varied

Figure 5. Method transfer from UltiMate 3000 to Vanquish system. Still air mode on Vanquish boosts efficiency, but reduces retention and selectivity.

Figure 6. Comparison as in Figure 5, but lower active pre-heater temperature to compensate frictional heat (best efficiency, selectivity and resolution).

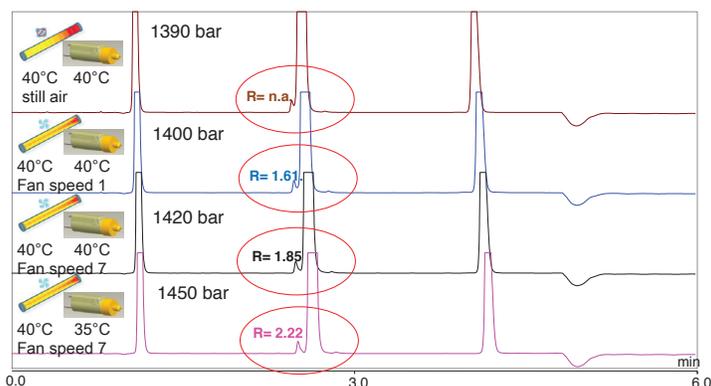


Experiment 4: Variation of Vanquish Column Thermostat Fan Speed and Active Pre-heater Temperature to Tune Internal Column Temperature for Best Resolution of an Impurity in the Presence of Excess Compound

A customer method showed a critical separation of a shoulder to the peak of a main component (caffeic acid). A 1.9 μ m stationary phase packed in 200 mm column length was selected to improve resolution of this impurity. It turned out that the selectivity was temperature dependent. The selectivity increased with the fan speed setting which increases heat distribution as can be concluded from elevated column pressure indicating the viscosity increase. In addition, to the elevated fan speed of 7, the active pre-heater temperature was set 5 °C below the compartment temperature which eventually increased the resolution to the excellent level of 2.22.

This example demonstrates how the properties of the Vanquish column thermostat can be used to adapt non-prescribed method parameters (fan speed, active pre-heater temperature) in order to fine tune a separation which would otherwise show poor resolution on still air thermostats due to temperature effects that result from frictional heat.

Figure 7. Setting of 3 different fan speeds and 2 different active pre-heater temperatures to tune the resolution of a shoulder peak to the main component caffeic acid. The method generates significant frictional heating and strong dependence of heat dissipation (see pressure) and selectivity (see resolution) on thermostat settings.



CONCLUSIONS

- Still air thermostating improves efficiency under frictional heating, but also changes retention relative to forced air thermostating. This implies challenges with method transfer between different column thermostat types.
- Change of thermostating mode or column compartment fan speed changes method selectivity under frictional heating when $\alpha = f(T)$. Lowering column eluent pre-heater temperature to compensate viscous heating effects in still air mode enables holistic selectivity and efficiency optimization. This specific setting of pre-heater temperature can also help in method transfer.
- The Vanquish column compartment is the only integrated column thermostat that allows switching between still air and forced air mode as well as setting a fan speed and independent active pre-heater temperature by the user. This opens up unique possibilities for method optimization and method transfer with challenging separations.

REFERENCES (if necessary)

1. Fabrice Gritti, Georges Guiochon, Anal. Chem 80 (2008) 5009-5020.
2. James P. Grinias, Dayley S. Keil, James W. Jorgenson, J. Chrom. A 1371 (2014) 261-264.

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