The Role of Temperature and Column Thermostatting in Liquid Chromatography

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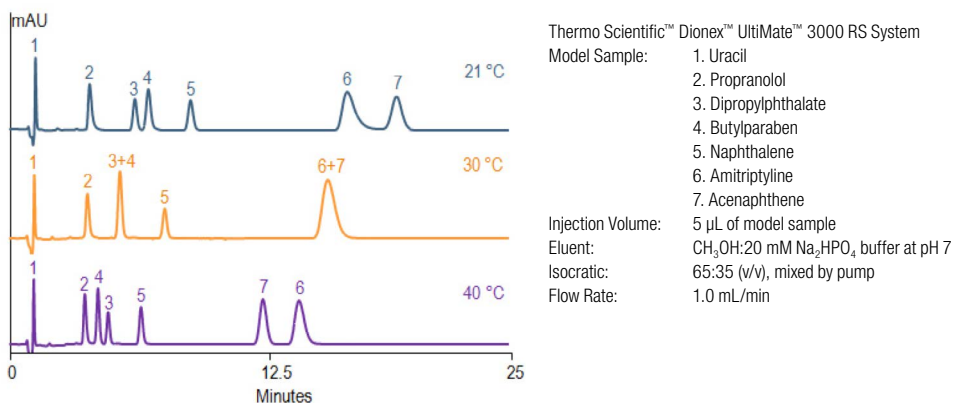
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Key Words

Column Compartment, Thermostatting Principle, Mobile Phase Pre-Heating, Thermal Mismatch, Frictional Heat, Post-Column Mobile Phase Cooling

Introduction

The use of elevated temperature in HPLC has a similar promise as using smaller particles with UHPLC—better performance and reduced analysis time (Figure 1). If the column temperature is increased, the chromatographic separation process becomes faster and, in general, more efficient. However, 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 (see Figure 1). Consequently, a proper column temperature control is essential for LC separations with marginal resolution of the critical peak pair or if retention times are used for the identification of individual sample compounds.

Figure 1. Stacked chromatograms of the same model sample separation at different temperatures.

Thermo Scientific™ Dionex™ UltiMate™ 3000 RS System

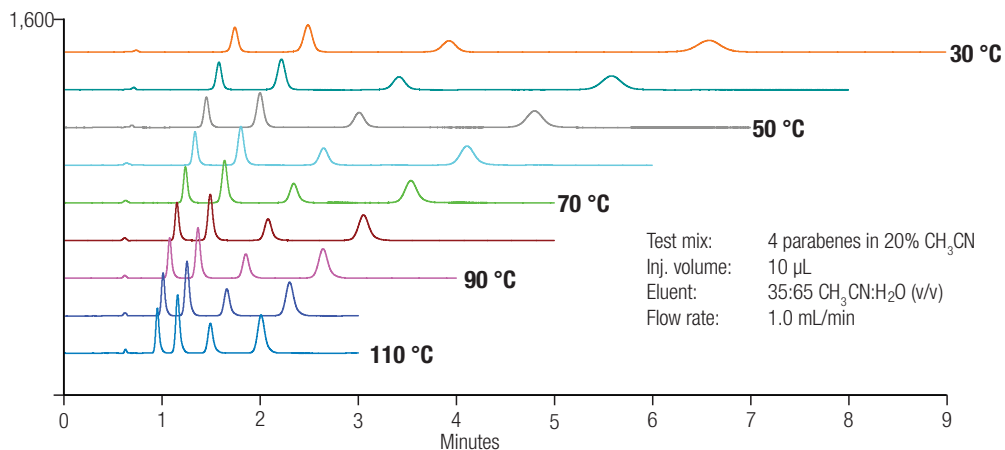|	
Model Sample:	1. Uracil
	2. Propranolol
	3. Dipropylphthalate
	4. Butylparaben
	5. Naphthalene
	6. Amitriptyline
	7. Acenaphthene
Injection Volume:	5 µL of model sample
Eluent:	CH_3OH:20 mM Na_2HPO_4 buffer at pH 7
Isocratic:	65:35 (v/v), mixed by pump
Flow Rate:	1.0 mL/min

Why is Temperature Control so Important in Liquid Chromatography?

If the column temperature is increased, the chromatographic separation process becomes faster. A rule of thumb for reversed-phase isocratic separation predicts a retention time decrease of 1–2% for each 1 °C column temperature increase. 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 (see Figure 1). Consequently, a proper column temperature control is essential for separations with marginal resolution of the critical peak pair or if retention times are used for the identification of individual sample compounds.

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When are Temperatures Above Ambient Used in LC?

A shorter run time is the main goal when the separation temperature is increased. With neutral sample compounds, this goal is achieved without much method rework as selectivity changes do not occur very often (see Figure 2). With polar and, especially, ionic sample compounds, selectivity changes are observed (see Figure 1) and method revalidation is needed in many cases. A lower system backpressure is another goal with a temperature increase. As the column temperature rises, the mobile phase viscosity decreases, thereby the flow resistance in the column, and the system backpressure as well.

Figure 2. Shorter analysis time through increasing the separation temperature.

Test mix: 4 parabenes in 20% CH_3CN
Inj. volume: 10 µL
Eluent: 35:65 CH_3CN:H_2O (v/v)
Flow rate: 1.0 mL/min

Both, lower mobile phase viscosity and increased temperature improve diffusion during the chromatographic process. Narrower peaks most often result, also depending on the applied flow rate of the mobile phase (cf. Van Deemter equation). A narrower peak indicates higher separation efficiency. This narrower peak is also taller, in comparison to its equivalent at the lower temperature, resulting in a lower detection limit. Furthermore, if the backpressure at the lower temperature had already been in the operational optimum of the liquid chromatography (LC) system, the run time can be shortened further by increasing the mobile phase flow rate until the original backpressure is reached and by adjusting the mobile phase gradient to this new flow rate.

In summary, increasing the separation temperature is used to shorten the analysis time, to improve separation efficiency, to achieve a lower system backpressure, or, in special cases, to obtain alternative selectivities with polar sample compounds (Figure 1).

What Types of Column Compartments Exist on the Market?

Different types of column thermostatting designs and concepts exist on the market. Most LC systems already include a dedicated column compartment. Generally, such a thermostat is either based on a block heater or air bath concept. With a block heater, the column is directly thermostatted by the heat source through effective thermal conduction of a grooved metal block or a flexible heating tape in which the column fits accurately. The direct contact between column and heat source provides efficient real-time column wall heating.

An air bath is less effective at controlling the column temperature in real-time. In comparison to metal, air is a very bad heat conductor. Hence, column temperature equilibration takes much longer with a still air thermostat than with a block heater. Next to this, a still air thermostat cannot react fast on temperature changes coming from the inner core of the column. Circulating air systems enforce the transfer of heat from source to column wall by forced convection. The effectiveness of column heating is controllable by the fan speed of the forced air thermostat.

State-of-the-art column thermostatting concepts use Peltier elements as the heat source. Such a column Compartment also provides fast and efficient column cooling next to heating. These column compartments allow the user to work in a wider temperature range, starting from 15—20 °C below ambient air temperature up to values between 70 °C and 130 °C.

What Does Thermostatting Principle of a Column Compartment Mean?

With a block heater, the column wall is thermostatted in real-time and very efficient. Hence, at least the column wall is kept constant at the set column compartment temperature. If the mobile phase that enters the column has a different temperature compared to the set column compartment temperature, the block heater forces the column wall not to change its temperature much. Consequently, the column wall acts as a heat exchanger and tempers the mobile phase within the column wall region. A column wall within a block heater shows a quasi-isothermal behavior as there is minimal temperature variation. Due to practical limitations in real life a system can never be 100% isothermal or adiabatic, however for readibility we will omit the quasi in future references.

A column wall is thermostatted indirectly and inefficient with a still air thermostat. A column slowly reaches the set compartment temperature. If mobile phase enters the column with a different temperature, the still air thermostat is not able to keep the column wall at the set column compartment temperature. The still air thermostat behavior is called quasi-adiabatic as heat exchange through the column wall happens, if at all, to a very low extent. Almost the entire column adopts the temperature of the mobile phase, depending on the flow rate of the mobile phase. Hence, mobile phase pre-heating is mandatory with still air thermostats.

Forced air thermostats provide an accelerated heat exchange by forced convection of circulating air. The faster the airstream circulation, the higher the heat exchange between heat source and column wall. The column wall thermostatting is, however, less efficient than with a block heater. Nevertheless, a column wall within a forced air thermostat shows a more isothermal than adiabatic behavior. When the mobile phase has a different temperature, the column wall changes its temperature a bit but stays close to the set column compartment temperature.

When Does Mobile Phase Pre-heating Play an Important Role?

Pre-heating of mobile phase becomes essential as soon as the optimum separation efficiency needs to be achieved (see Figure 3). The extra-column volume (ECV) of a passive pre-heater is a certain drawback as it can affect separation efficiency as well, especially with micro-bore LC columns (1 mm I.D.). Active pre-heaters do not contribute to the ECV as they are, typically, placed on a system capillary that would have been used in that length and diameter anyway.

Figure 3. Shorter analysis time through increasing the separation temperature.
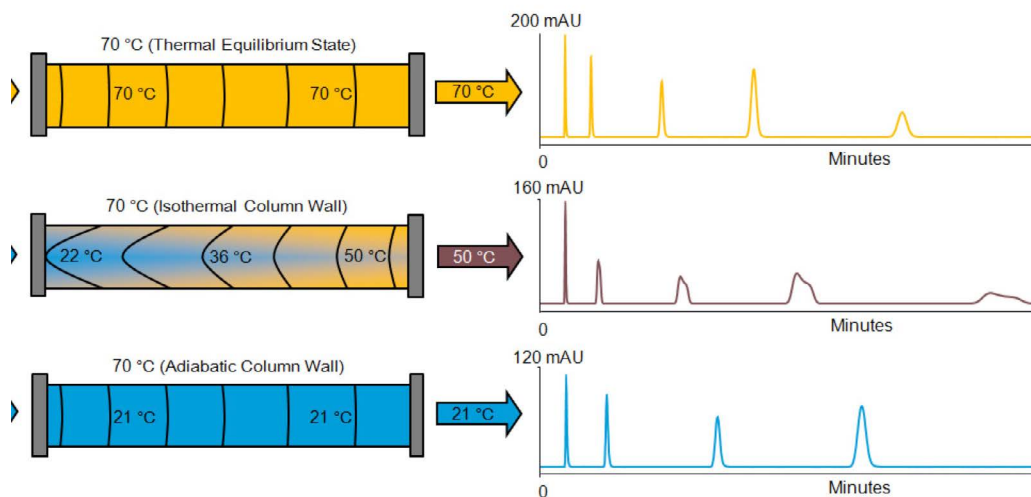
Thermo Scientific™ Vanquish™ UHPLC System
Model Sample: 1. Uracil
            2. 4-Nitroaniline
            3. Methyl Benzoate
            4. Phenetole
            5. o-Xylene
Inj. Volume: 5 µL of model sample
Eluent: 40:60 $CH_3CN:H_2O$ (v/v)
Flow Rate: 1.4 mL/min

Note: To achieve adiabatic conditions, the column was placed inside a plastic tube within the Vanquish Column Compartment H. For isothermal conditions, the column was wrapped tightly with aluminum foil until it stuck in the Vanquish Column Compartment H without any additional interlock. The pre-heater was not actively used except for the case of thermal equilibration state.

When a isothermal column thermostat is used, colder incoming mobile phase is re-heated along the tempered column wall while the mobile phase in the center of the column keeps its colder temperature for a certain period of time until it also heats up. This radial temperature gradient results in a significant thermal mismatch within the column. Then, the viscosity of the mobile phase along the wall is lower to that in the center of the column. Different mobile phase flow rates along the column wall and its center occur. Loss of separation efficiency and even distorted peaks result (see Figure 3).

With a adiabatic column thermostat, the column wall is cooled down by the mobile phase and is re-heated very slowly by the thermostat. Depending on the flow rate, the entire column can adopt the mobile phase temperature (see Figure 3). Hence, the chromatographic separation occurs at the mobile phase temperature but not at the set compartment temperature. State-of-the-art column thermostatting concepts include a mobile phase pre-heater, which supports a proper column heating with still air thermostats.

What Does Frictional Heat Mean?

It is a matter of fact that small particle stationary phases generate significant heat by viscous friction of the mobile phase, especially under ultra high column backpressures. A column compartment that keeps the column wall isothermal enables efficient removal of frictional heat and avoids a pronounced temperature increase inside the column. As the generated heat dissipates from the column center to the column wall, this heat removal induces a radial temperature gradient inside the column (see Figure 4).

In such a case, the column center is hotter than the column wall. Due to the cooperative effect of temperature induced lower retention and, secondarily, viscosity induced higher linear velocity, the analyte molecules move much faster in the column center than closer to the column wall where temperatures are lower. This leads to peak broadening and results in a loss of separation efficiency. In some cases, peak distortion is also observed (similar to Figure 3).

If the heat exchange between column wall and the column compartment is suppressed, as it is with adiabatic still air thermostats, the frictional heat stays inside the column. Hence, the temperature constantly increases axially from column inlet to column outlet as the heat is transported in flow direction. At the same time, radial temperature gradients are eliminated and peak distortion through thermal mismatch is suppressed.

Figure 4. Effect of frictional heating on the temperature distribution inside a column and chromatogram.
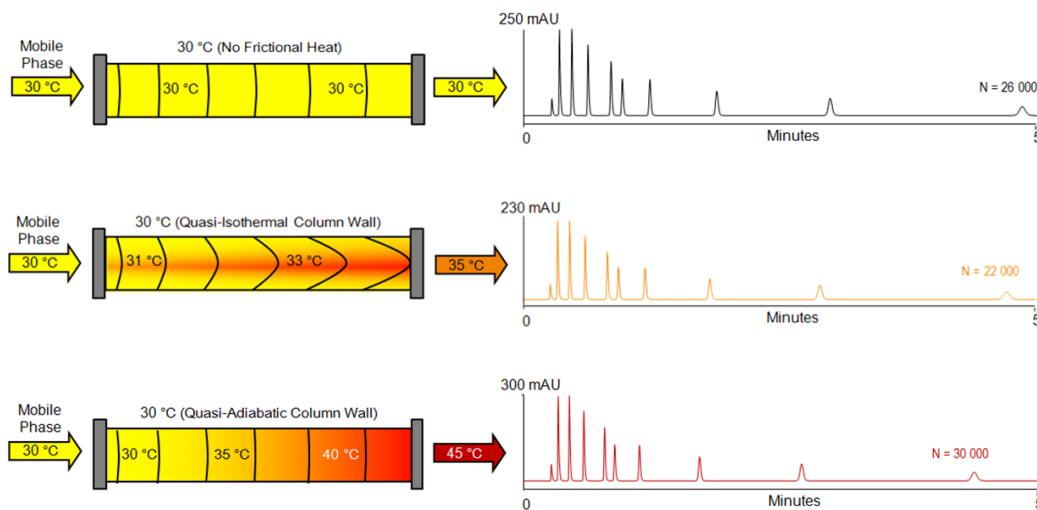
Vanquish UHPLC System

Model Sample:	Uracil, acetanilide, and 8 phenones
Injection Volume:	1 µL of model sample
Eluent:	55:45 $CH_3CN:H_2O$ (v/v)
Flow Rate:	0.65 mL/min

Note: For isothermal conditions, a forced air mode with the highest settable fan speed was applied. For adiabatic conditions, the still air mode was applied. To mimic the "no frictional heat" situation, the mobile phase had an inlet temperature of 23 °C and heated up by friction to 39 °C. At least, this temperature was measured for the mobile phase that eluted from the column. Hence, an average separation temperature of 30 °C can be assumed giving an equal result as with the thermal equilibration state at 30 °C.

Which Considerations are Important Regarding Frictional Heating Effects?

In general, the effect of frictional heating is negligible with conventional HPLC operated at a column backpressure below 300 bar. However, frictional heat becomes significant at column backpressures greater than 400 bar. The higher the column backpressure, the higher the frictional heating inside the column. When frictional heat appears inside a column, still air thermostats induce larger axial temperature gradients while block heaters induce larger radial temperature gradients. With forced air thermostats, the proportion between the axial and the radial temperature gradient is controllable by adjusting the fan speed. If the fan is turned off, the same axial temperature gradient is received as with a still air thermostat.

A radial temperature gradient has a greater influence on the flow profile within the column and the separation efficiency than an axial temperature gradient. An axial temperature gradient alters retention times and, in some cases, selectivity because the average separation temperature is increased. The column efficiency, in turn, is not badly affected. Most UHPLC systems include a still air thermostat and, hence, the column is operated under adiabatic conditions. Then, retention times and selectivities are prone to change according to the frictional heat amount inside the column. This is critical for method transfer when the average separation temperature becomes different between the original HPLC method and the related UHPLC method. At typical UHPLC pressures, frictional heat contributes much to the average separation temperature which is, consequently, much higher than the set compartment temperature and the average separation temperature of the HPLC method as well.

When using a mobile phase temperature being to that extent lower that it balances out the frictional heat contribution, the average separation temperature is the same as with the HPLC method. In that case, same retention factors and selectivities result with the HPLC and UHPLC method. A similar approach is the use of a lower column compartment temperature with the UHPLC method. Then, same average separation temperatures are achieved with the original HPLC and related UHPLC method as well. Both approaches require UHPLC method rework.

The separation efficiency loss by a radial temperature gradient becomes less pronounced with decreasing column diameter. When using a forced air thermostat or a block heater with high efficient narrow bore or micro-bore UHPLC columns packed with sub-2 µm particles, the efficiency loss by radial temperature gradients can be more acceptable than the change in retention time and selectivity by an axial temperature gradient where a still air thermostat would be used instead. UHPLC method rework is most often not necessary with this approach.

Which Limits Exist When Working at Temperatures Above Ambient?

Very temperature-sensitive sample compounds are likely to alter with each temperature increase until they decompose. This can also happen to some mobile phase additives. The high pressures in the LC system allow mobile phases to be used well above their normal boiling points. The critical temperature and pressure define the upper limit of operation. Nevertheless, most LC columns should not be operated at temperatures above 60–70 °C for a long period of time. At elevated temperatures of 80–90 °C, the silica backbone of the stationary phase or its bonding are prone to decompose, resulting in column bleeding.

Next to this, the vapor pressure of the solvent rises with increasing temperature and vapor bubbles might outgas. When running separations at elevated temperatures above 60 °C, boiling of high organic mobile phases might appear in the detector. This happens when the mobile phase is heated beyond its boiling point at ambient pressure. During the separation, boiling of mobile phase was suppressed by the high system pressure. Increased baseline noise, ghost peaks or even complete light absorption can appear with column bleeding, with vapor bubbles, or with mobile phase boiling in the detector flow cell. Next to this, some flow cells are also susceptible to temperatures above 60 °C and can be damaged.

The use of a post-column mobile phase cooler maintains a sufficient mobile phase temperature for proper detector operation, at the latest when working at elevated separation temperatures above 60 °C. After a low-temperature separation, it is also very reasonable to warm up the mobile phase in front of an optical detector to prevent moisture condensation on the optics. Hence, a post-column heat exchanger, which can also heat the mobile phase, becomes important. In general, a post-column mobile phase cooler or heat exchanger should be used as long as its extra-column volume does not affect the separation efficiency measurably.

Conclusions

The average separation temperature determines retention times, selectivity, efficiency, and resolution of a liquid chromatographic separation. The set column compartment temperature and the real separation temperature inside an LC column deviate from each other as soon as the mobile phase is not properly pre-heated or when frictional heat that occurs at higher column back pressures is not taken into account. Column compartments have to be distinguished into different thermostatting principles like isothermal or adiabatic. A isothermal column compartment, with block heaters and forced air thermostats being the most important variants, induces radial temperature gradients within the LC column if mobile phase and compartment temperature are not equal. The source of this thermal mismatch could either be a colder or hotter (e.g., by frictional heating) mobile phase in comparison to the column wall. Radial temperature gradients inside an LC column lead to peak broadening, even peak distortion and separation efficiency loss while retention times and selectivities of the sample compounds are less affected.

A adiabatic column compartment, with the still air thermostat being the only variant, is dominated by the actual mobile phase temperature. Depending on the flow rate, the entire LC column can adopt the temperature of the mobile phase instead of the set compartment temperature. When frictional heat appears, the still air thermostat induces an axial temperature gradient within the LC column. The average separation temperature increases, retention times and selectivities of the sample compounds might change while the separation efficiency does not suffer. Quite contrary, the separation efficiency can increase due to the higher separation temperature, depending on the applied flow rate.

A post-column mobile phase cooler or, even better, heat exchanger with the possibility to heat and cool the mobile phase in front of the detector cell is essential as soon as sub-ambient or elevated temperature LC methods are used or when frictional heat is prone to heat the mobile phase above its boiling point at ambient pressure.

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