

# An Introduction to Elevated Temperature in HPLC

## *Theory and principles for elevated temperature and temperature programmed liquid chromatography*

The SandraSelerity Polaratherm is designed to enhance the analysis of chemical mixtures through HPLC at elevated and sub-ambient temperatures. Although often neglected, temperature plays an important role in HPLC since the majority of the chromatographic properties are a function of temperature. Nevertheless, to this date the possibilities of temperature to improve LC separations have not been fully investigated and the majority of liquid phase separations are performed in the temperature region between 20 to 40°C or without thermostating.

The use of elevated temperature has proven effective for improving the overall chromatographic performance on conventional equipment.

The main advantages of using temperature in LC are:

### **1. Speed**

Increase speed by increasing the temperature and flow rate.

### **2. Efficiency and resolution**

Increase efficiency by using longer columns/smaller particles at elevated temperature.

### **3. Selectivity**

Change selectivity with temperature.

### **4. Lower consumption of organic solvents - Green chromatography**

Higher temperature requires less solvent.

### **5. Improved detectability**

Improve peak shape. Use only water.

### **6. Temperature programming**

Replace solvent gradients with a temperature program.

## 1. Speed

The current trend is to use shorter columns with smaller particle sizes, typically less than 2  $\mu\text{m}$ , for increasing the speed. This has the disadvantage that the pressure drop over such columns is drastically increased. Furthermore, to fully exploit the potential of such 'sub-two-micron' columns high linear velocities have to be used. The combination of the intrinsic high backpressure and the required high flow rate causes analysts to reach the upper pressure limits of conventional LC systems. The recent introduction of ultra-performance or ultra high pressure LC (UPLC) equipment has partially overcome this problem.

In nearly all reversed phase separations, an increase in temperature will also cause a decrease in retention. Additionally, decreased solvent viscosity at elevated temperature (1-2% per  $^{\circ}\text{C}$ ) leads to lower back pressure. This allows the use of higher flow rates using standard equipment. Since high temperature leads to a flatter van Deemter curve, it enables the use of higher flow rates without hampering efficiency (Figure 1). An increased flow rate is even favorable to fully benefit from the increased temperature.

The application of high temperature enables the use of columns packed with 'sub-two-micron' particles at high flow rates. In this way it is possible to exploit the advantages of these columns using conventional LC equipment.

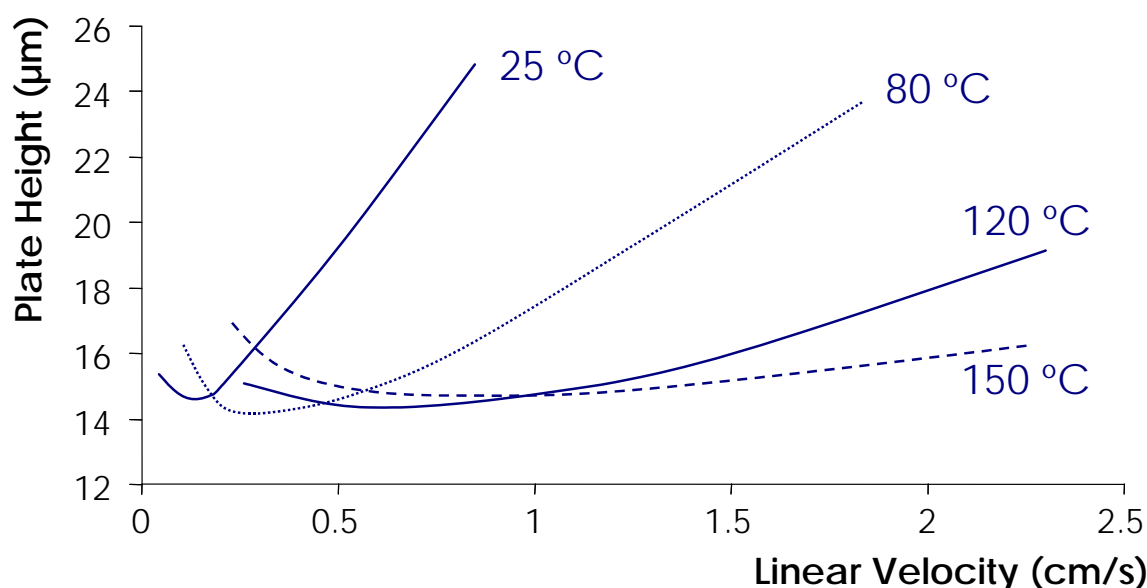


Figure 1. Plate height versus linear velocity.

## 2. Efficiency and resolution

The efficiency that can be realized on one particular analytical setup is relatively independent of analysis temperature provided that adequate preheating of the mobile phase prevents radial temperature gradients. At elevated temperature however, the solute transfer from the mobile phase to the stationary phase is more efficient. This results in high efficiency, even at elevated flow rates.

The low back pressure allows the use of smaller particle sizes and/or longer columns to increase efficiency and resolution.

This also opens perspectives for increased efficiency and resolution in preparative LC. Instead of multiple collection sessions to completely resolve all of the compounds, a single method with increased resolution can provide a more elegant and productive alternative.

In practice, conventional LC columns can be coupled in series and operated at elevated temperature to decrease the backpressure (e.g. four 25 cm columns result in a total column length of 100 cm). Since the column plate number is determined by column length, efficiency will increase proportionally with column length.

### 3. Selectivity

The stationary phase, mobile phase pH, organic modifier, and several other parameters determine selectivity in LC. Temperature is a parameter that also plays an important role in this aspect. This is especially true for polar and ionizable compounds since ionization equilibria are temperature dependent. Even more, because it is an instrumental setting, temperature is one of the easiest and most straightforward parameters to change and control in order to tune chromatographic selectivity. This has been reported frequently in the literature, yet its potential role in method development is still often underestimated.

### 4. Lower consumption of organic solvents - Green chromatography

By increasing the temperature, the amount of organic solvent in the mobile phase can be reduced to maintain retention. Roughly, a temperature increase of 4 or 5°C has a similar effect on retention as a 1% increase in methanol or acetonitrile, respectively. Superheated water at 200°C has a similar eluting power as methanol at ambient temperature. Additionally, since the back pressure is reduced at elevated temperature, ethanol becomes a practical alternative for toxic solvents such as methanol and acetonitrile. Mobile phases composed of water, ethanol, and additives like ammonia and acetic acid can be considered non-toxic and can be used for green chromatography.

### 5. Improved detectability

The reduced amount of organic solvent in the mobile phase also results in additional advantages. The mobile phase UV transparency in the low UV range is improved. Alternative detection techniques such as FID (Flame Ionization Detection) also become an option when using solvent-free mobile phases.

Additionally, an improved peak shape for basic solutes is frequently encountered. The ionic strength and buffer pH required for good peak shape of these solutes can therefore often be changed to levels less harmful for the column and chromatographic system.

## 6. Temperature programming

Since the temperature of the column and mobile phase influences the retention, programming the temperature in time can be used to elute compounds from the column. If the system is capable of covering large temperature ranges, a temperature gradient can be used as in gas chromatography and in many instances, can replace the solvent gradient. This enables the use of temperature programmed elution on detectors that are restricted to isocratic operation such as a refractive index detector.

## Requirements for high temperature and temperature programmed HPLC

Successful implementation of HPLC at elevated temperatures depends on these key elements:

- ❑ Preheating the mobile phase to avoid band broadening related to thermal mismatch across the column
- ❑ Ability to efficiently heat both the exterior space around the column and the fluid entering the column to allow rapid temperature programming.
- ❑ Columns that are stable at elevated temperatures.

The SandraSelerity Polaratherm incorporates a forced air column compartment with the capacity to hold a wide range of column types, a high performance mobile phase preheater that efficiently preheats the mobile phase just prior to column entry, and a fast cooling device that uses a Peltier chip for precise temperature control of the column effluent. The Polaratherm contains a sensor that automatically initiates an instrument shut-off procedure upon detection of flammable vapors.

### Column stability

When using water in the mobile phase as in most reversed phase type separations, loss of the bonded phase from the silica support due to hydrolysis is enhanced at high temperatures. Therefore, traditional silica-based stationary phases usually are stable at temperatures up to 60°C and in some instances up to at least 90°C (e.g. Zorbax StableBond C18 from Agilent Technologies, XBridge BEH from Waters).

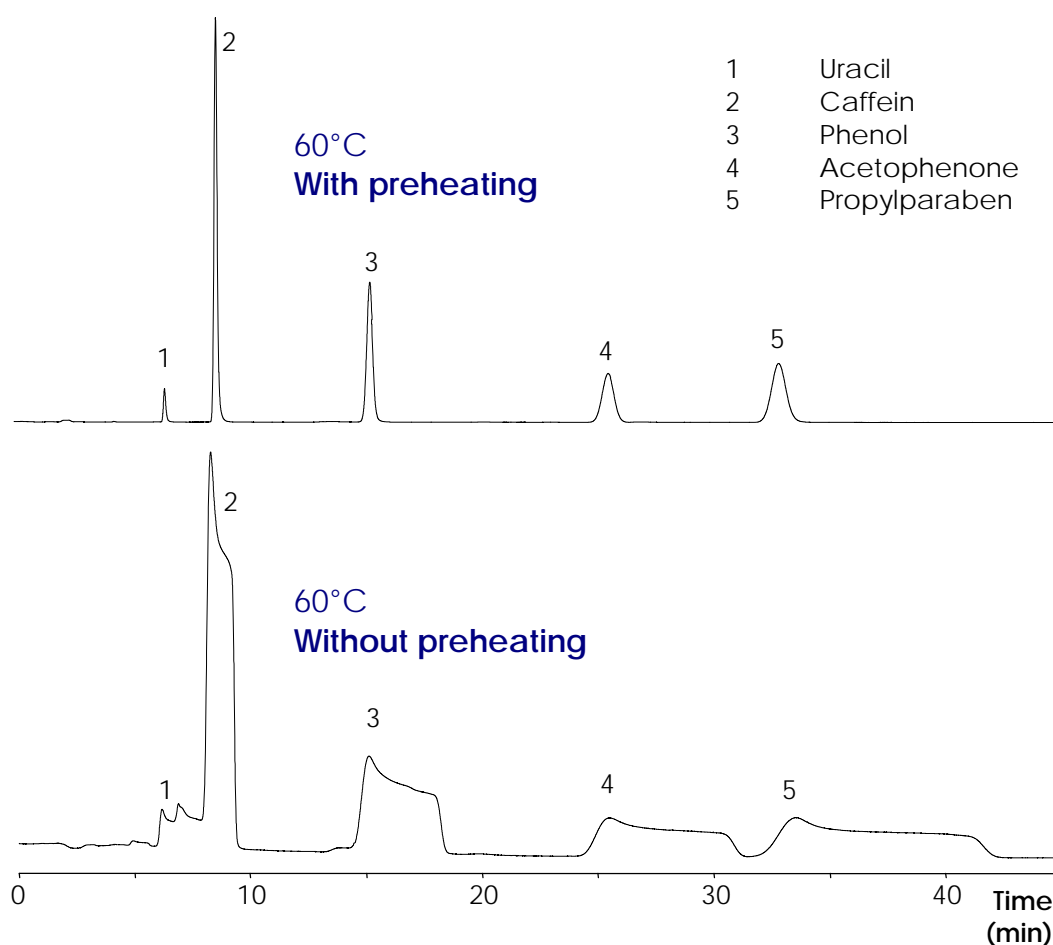
At this time, new temperature stable silica-based columns (Blaze200 from Selerity Technologies) and alternative stationary phases are available which enable the application of high temperature over a long period of time while maintaining column performance.

Stationary phases with the highest temperature stability are based on materials other than silica e.g. graphitized carbon types, zirconium oxide based phases and polystyrene/divinylbenzene phases. For columns intended to be used at temperature of 120°C or higher, care has to be taken that the PEEK present in the column hardware is replaced by stainless steel.

Columns should be used only within the rated specifications and guidelines provided by the vendor. Using a column outside of its recommended temperature, mobile phase or pH range can result in rapid degradation of the column. This can cause irreversible damage to the column, and lead to the production of particulates that may plug the lines and detector components of the HPLC system.

### Thermal mismatch

Thermal mismatch is defined as the difference in temperature between the column and the mobile phase, producing a temperature profile across the column radius. Therefore, performing LC at elevated temperature requires accurate control of the column temperature and the incoming mobile phase. Thermal mismatch causes the sample front to distort resulting in band broadening, poor peak shape, and split peaks. An example of this effect is shown in Figure 2.



**Figure 2.** Comparison of LC separation at 60°C with and without mobile phase preheating.

The temperature of a column can be controlled in several ways using e.g. heating blocks, water jackets and baths, and circulating air ovens. The main problems with water jackets and baths are the limited temperature gradient rates that can be achieved by such a system. The efficiency of heating blocks depends largely on the degree of contact with the column hardware. This setup also suffers from limited temperature gradient rates. Circulating air ovens have a heating capacity that mainly depends on the speed at which the heated air can be blown around the column. The main advantage is that the temperature can be varied relatively fast.

The SandraSelerity Polaratherm allows elevated temperature and temperature programmed LC with conventional columns and HPLC equipment. The system actively heats the entering mobile phase to the same temperature as the column/oven temperature. In this way, loss of separation and efficiency due to thermal mismatch between the column wall and the incoming mobile phase and column centre is eliminated. Furthermore, the temperature can be set equal, higher or lower to the column temperature.

## Method Development

### To develop a new method

Choose an isocratic mobile phase. The type of column being used and nature of the analyte should be considered. Polymeric columns generally require a higher concentration of organic modifier than silica or zirconia based columns. The polarity, functionality, structure, pKa and reactivity of the analyte must also be considered. The amount of organic modifier in the mobile phase can generally be reduced by about 10-20% at 50°C when compared to room temperature.

Choose the proper mobile phase buffer. In many cases, water without a buffer will provide an adequate separation. Selection of mobile phase pH should be based on the pKa of the analyte of interest. There are several good sources for information on obtaining pKa information and selecting mobile phase buffers.

Run a temperature gradient from 35°C to the maximum recommended temperature for the column you are using. Hold at the maximum temperature for five minutes. Use a ramp rate of 10°C or 15°C per minute.

Based upon the results, decide if you want an isothermal or a temperature gradient method. For an isothermal method, optimize temperature, and adjust the mobile phase composition to achieve the best separation. Optimize the flow rate to achieve the shortest analysis with adequate resolution and efficiency. For a temperature gradient method, choose a starting temperature and adjust the mobile phase composition, then optimize ramp rate and flow rate.

### To replace an existing binary solvent gradient with a programmed temperature gradient

Choose an isocratic mobile phase composition approximately midway between the starting and ending compositions of the solvent gradient. For example, if you are running a linear gradient from 20% to 100% acetonitrile, start with an isocratic mobile phase of 40% water and 60% acetonitrile.

Run a temperature gradient from 35°C to the maximum allowable temperature for the column you are using. Hold at the maximum temperature for at least five minutes to ensure elution of all components. Use a ramp rate of 10°C or 15°C per minute.

Based on the results, choose a starting temperature then adjust the mobile phase composition as necessary. Optimize ramp rate and flow rate to achieve the shortest analysis time with adequate resolution and efficiency.

### To speed up an existing isocratic method or reduce the amount of organic modifier in the mobile phase

Start with the existing isocratic method. Perform the analysis at 50°C. Increase the temperature in 50°C increments, reducing the amount of organic modifier in the mobile phase as necessary to produce a good separation. Optimize the temperature, mobile phase composition and flow rate to achieve the shortest analysis time with adequate resolution and efficiency.