The Use of Temperature for Method Development in LC

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The use of temperature as a tool for method development is gaining interest. In most of the reversed-phase LC methods the selectivity can be altered significantly by changing the temperature. Since temperature is an instrumental parameter it is easier to change than e. g. buffer pH and is more robust. This note demonstrates the potential of temperature variations for controlling the selectivity in LC method development. Additionally, the features of a silica based and a polymeric stationary phase for elevated temperature LC are highlighted.

Increasing the column temperature has several advantages amongst which the gain in speed and efficiency and the possibility to alter the chromatographic selectivity are some of the most important.

Speed & Efficiency

An increase in temperature will generally cause a decrease in retention. The reduced solvent viscosity at elevated temperature will lead to lower back pressure and allows the use of higher flow rates and/or smaller particles to increase the analysis speed using standard HPLC equipment. The low back pressure at elevated temperature also permits to use longer columns combined with higher flow rates to increase the efficiency and resolution without significantly raising the analysis time.

At elevated temperature the solute transfer from the mobile phase to the stationary phase is more efficient (C-term in van Deemter equation). The result is a flatter van Deemter curve. This enables, and even requires, using higher flow rates without sacrificing efficiency. For reversed-phase separations on silica based columns an increase in temperature will not significantly affect the minimum plate height unless secondary interactions are prominent. Polymeric stationary phases suffer from a significantly slower diffusion rate of the solutes in and out the stationary phase, leading to lower efficiencies compared to their silica equivalent. By using elevated temperature the diffusion rates are improved and the viscosity of the mobile phase is reduced. Therefore, the plate height will decrease and efficiencies at high temperature will be comparable to efficiencies obtained with silica based reversed-phase columns.

This is demonstrated in Figure 1 where the reduced plate height (h) at various temperatures and flow rates is compared for a silica based column (Blaze200 C18, 15 cm L x 4.6 mm ID, 3 µm particles, Selerity Technologies, Salt Lake City, UT, USA) and a polymeric column (ET-RP1, 15 cm L x 4.6 mm ID, 5 µm particles, Shodex, Munich, Germany). The mobile phase composition was modified to keep the k-value nearly constant at ca. 1.8. It has to be noted that the silica based stationary phase that is used here is specially designed for use at high temperatures and that most commercially available phases should be used at temperatures below 70-80°C. The use of polymeric columns has been described by our group in reference [1].

Selectivity

Selectivity is the most important factor for resolving compounds in LC. There are numerous ways to alter selectivity. The most common in reversed phase LC are the

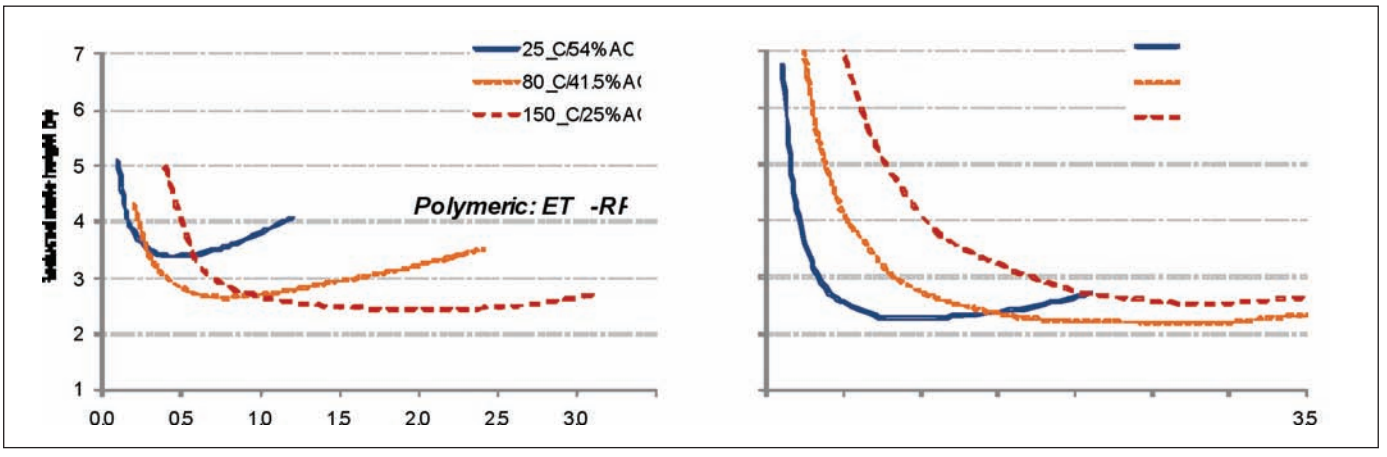

Figure 1. Van Deemter curves for acetophenone (200 µg/mL in water/acetonitrile 75/25 v/v) on a polymeric and a silica based column. Mobile phase: water/acetonitrile, injection volume: 2 µL, detection: DAD, 210 nm. Temperature is controlled by Polaratherm 9000 Series column oven equipped with active mobile phase preheating and effluent cooling (SandraSelerity Technologies, Kortrijk, Belgium).

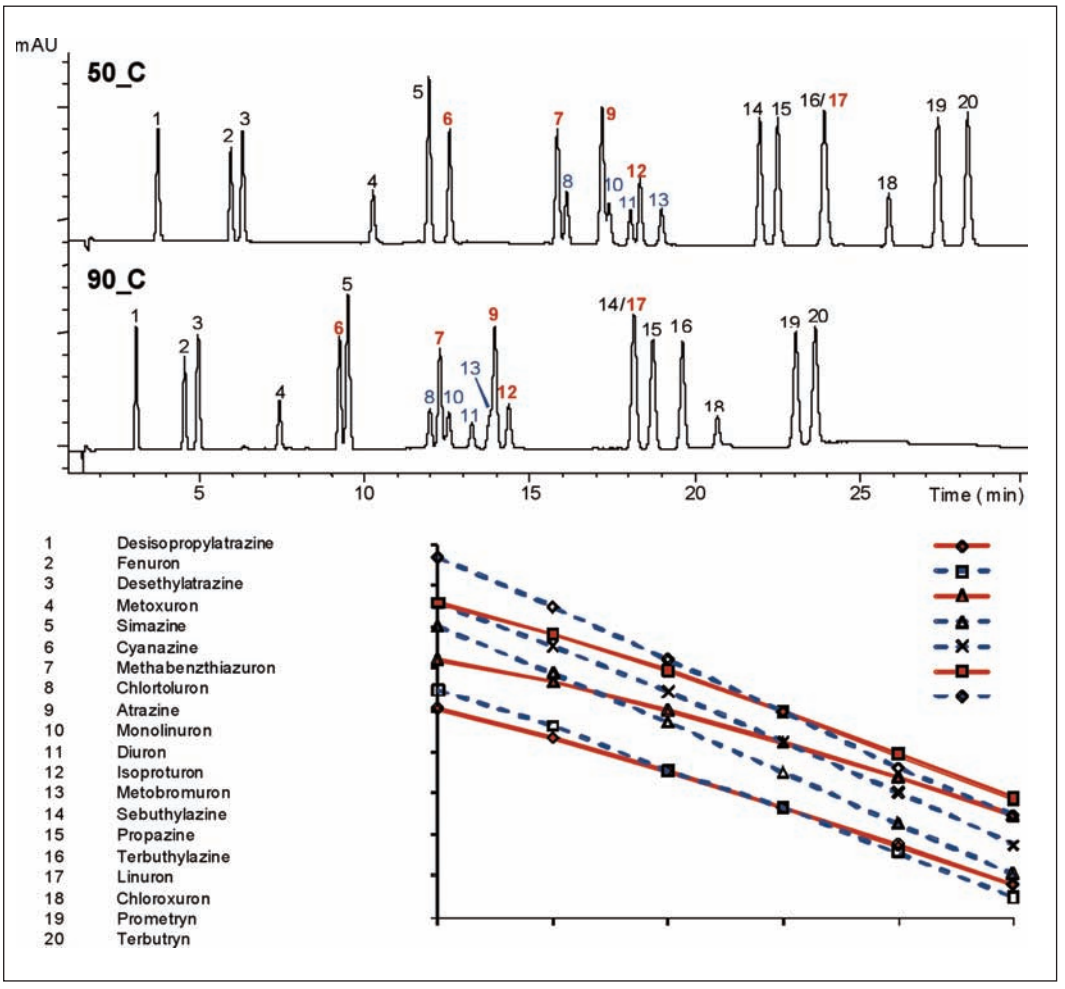

Figure 2. Influence of temperature on the analysis of a mixture herbicides on a Zorbax StableBond C18 column (150 mm L x 4.6 mm ID, 1.8 µm particles). Flow rate: 1 mL/min, gradient: water/ACN 80/20 to 45/55 in 30 min, detection: DAD, 230 nm. Temperature is controlled by Polaratherm 9000 Series column oven equipped with active mobile phase preheating and effluent cooling (SandraSelerity Technologies, Kortrijk, Belgium).

nature of the stationary phase and the composition of the mobile phase (buffer pH and composition, organic modifier type, additives etc). Since the ionization equilibria of analytes and mobile phase are temperature dependent, the retention behavior of polar and ionizable compounds, and consequently the selectivity, will be significantly affected by temperature variations. Although reported more than one decade ago and recently re-emphasized in the literature [2-5], temperature is often overlooked in this respect and is rarely investigated in depth during method development. Very often, temperature screening will be the last resort if the previously described variables do not deliver or it will be evaluated at the end of the method development process to fine-tune the resolution between critical pairs.

In order to fully exploit the influence of temperature on a separation, larger temperature windows (e.g. between 20 and 150 °C) have to investigated. When doing so, very significant selectivity changes and often reversal elution orders can be accomplished. Even so, at this moment high temperature LC is not a common approach in daily practice. One reason is that performing high temperature LC and temperature programmed LC on an analytical scale necessitates the use of dedicated equipment to preheat the incoming mobile phase and column and to cool down the column effluent before detection [5]. A second reason is that the range of columns that is compatible with high temperature is considerably smaller than the number of traditional columns. Very often, the high temperature rated columns (e.g. graphitized carbon, zirconium oxide based phases, polymers) are also very different in nature compared to the more common phases. This gives rise to very different selectivities which makes it much harder for the chromatographer to translate existing methods to high temperature methods.

However, several typical LC columns tolerate relatively high temperatures. An example is shown in Figure 2 where a set of 20 herbicides (phenylurea and triazines) is analyzed on a Zorbax StableBond C18 (Agilent Technologies, Waldbronn, Germany) at 50 and at 90°C. Major selectivity changes are seen in the chromatogram. A graph showing the retention time in function of temperature for the central region of the chromatogram is inserted in the figure.

The highest temperature in the previous analysis corresponds with the upper limit that is supported for this particular stationary phase. If higher temperatures or larger temperature windows need to be investigated, alternative column material has to be used. When using some polymeric stationary phases (e.g. ET-RP1), the temperature can be increased up to 150°C. Additionally, polymeric stationary phases often are chemically more resistant and inert. The combination of a large temperature and mobile phase pH window is extremely powerful in method development. Figure 3 shows an example of the analysis of a mixture of a pharmaceutical (Metoclopramide) and its documented impurities (European Pharmacopoeia, EP) on a polymeric ET-RP1 column (Shodex, Munich, Germany). The analysis was carried out at different temperatures with a mobile phase at pH 9. The temperature was varied between 50 and 125°C during method development and the final temperature was set at 105°C. It is clear that the performance of most traditional silica based columns would deteriorate fast when applying these conditions. This was not the case for the polymeric column where the performance was maintained after the complete validation of the high temperature method [1].

Conclusion

This technical note demonstrates that temperature is a useful parameter for method development in LC. Temperature can be actively employed as a selectivity tool and not merely as a final fine-tuning parameter.

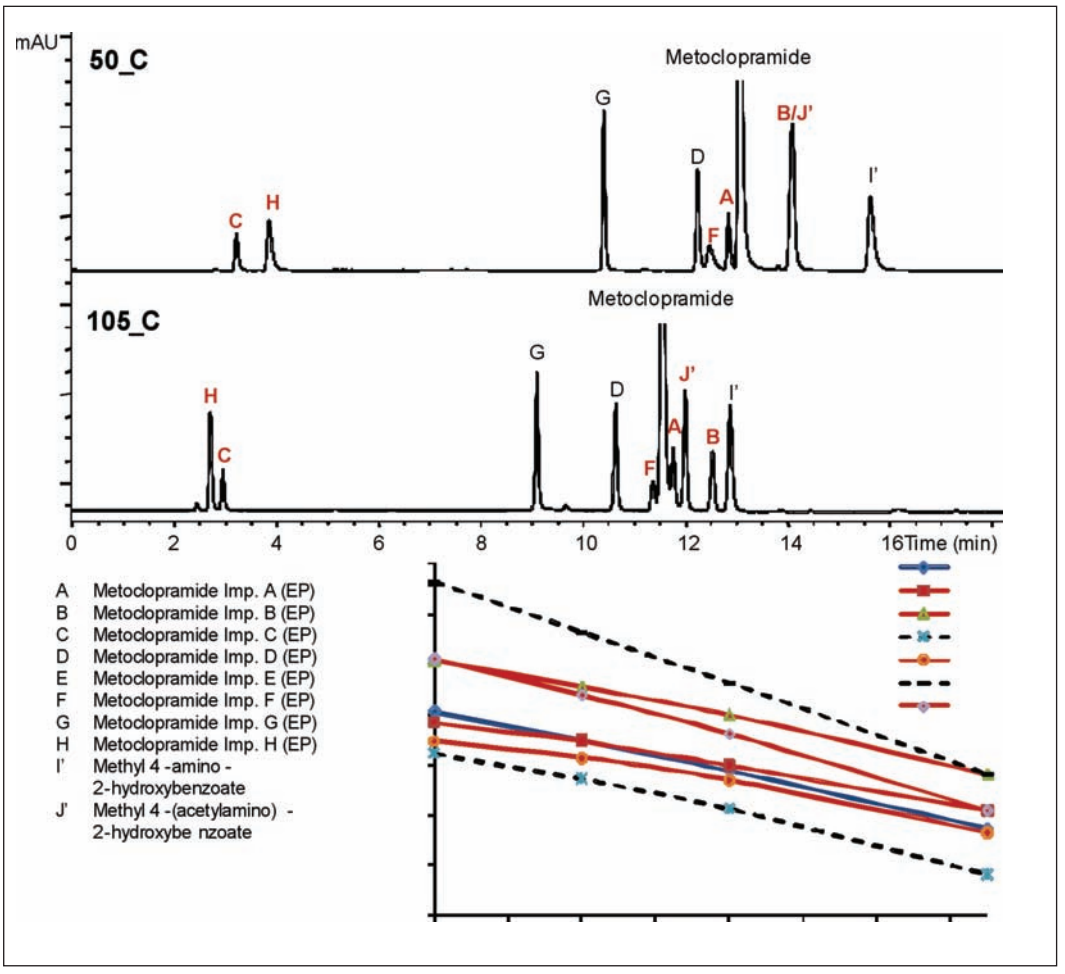

Figure 3. Influence of temperature on the analysis of a mixture Metoclopramide and impurities on a Shodex ET-RP1 column (150 mm L x 4.6 mm ID, 4 µm particles). Flow rate: 0.65 mL/min, gradient: ammonium acetate pH 8.8/ACN, 95/5 hold for 3 min, gradient 95/5 to 5/95 from 3 to 18 min, detection: DAD, 275 nm. Temperature is controlled by Polaratherm 9000 Series column oven equipped with active mobile phase preheating and effluent cooling (SandraSelerity Technologies, Kortrijk, Belgium).

Since temperature is an instrumental setting, it is one of the easiest and most straightforward parameters to tune chromatographic selectivity. Analyte stability is a major concern when using high temperature in LC. However, many compounds considered thermally labile do not degrade when being analyzed at higher temperatures. If degradation does occur, the amount of breakdown depends not only on the time spent at the higher temperatures, but above all on the nature and quality of the packing material (e.g. presence of trace metals) [5,6].

References

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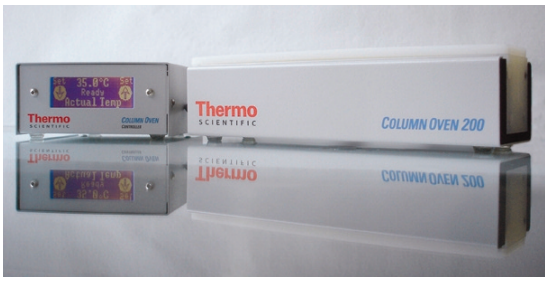
New Column Oven for Liquid Chromatography



Thermo Fisher Scientific announce the availability of the new Thermo Scientific Column Oven range. Recognizing the importance of temperature as a parameter in liquid chromatography, the Column Oven delivers efficient temperature control coupled with the highest levels of reproducibility and stability.

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Dani SpA and Jeol Enter Distribution Agreement

Dani SpA, a producer and developer of high level GC systems and attachments for the GC market and Jeol (Europe) BV have signed a distribution agreement for the Benelux countries. As the companies have complimentary systems and do not compete on any level, both Dr Umberto Saini from Dani and Dr Bob Hertsens from Jeol are looking forward to expanding market share with this strategic agreement, a report said. The Master-TOF high throughput GC-TOF developed by Dani can be combined with the JMS-T100GCv from Jeol for example and Q-MS was also said to be available. The companies are expecting that the combination of GC-TOF systems will open possibilities in applications such as fragrances, environmental, petrochemical and food research, as well as quality control and other areas.

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