High temperature liquid chromatography – a brief review about an emerging technique

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This review is focused to present a general overview about high-temperature liquid chromatography. It starts with a brief definition and then explains the necessary requirements to make use of this emerging technique. Also, the advantages of high-temperature liquid chromatography such as the reduction in the mobile phase's viscosity and the possibility to replace toxic organic solvents with water are outlined. Furthermore, the influence of temperature on selectivity is demonstrated. This means that temperature gradients can be integrated into method development to optimize the resolution of critical peak pairs.

In the last few years, there is renewed interest to explore the full potential of temperature in liquid chromatographic separations. Why is this? Although it might sound curious, temperature can be regarded as a universal parameter in liquid chromatography. Temperature influences almost every other parameter which can be used to optimize a separation in terms of speed and resolution [1,2]. However, this is only one aspect. There are some special hyphenation techniques which rely on the use of a water-only mobile phase [3-6]. In this case, temperature is the only option to change the solvent properties of water, which becomes more like an organic solvent with increasing temperature [7]. Before going into further detail, it is useful to define the practical temperature range in high-temperature liquid chromatography.

Until yet, a definition of high-temperature liquid chromatography does not exist although this technique has emerged as the topic of many scientific meetings and symposia. First of all, the lower temperature range needs to be defined. In this respect, the boiling point of the mobile phase should be considered. Methanol and tetrahydrofuran, which are widely used in reversed-phase liquid chromatography (RP-HPLC), start to boil at 65 and 66 °C, respectively. Hence, the domain of high-temperature HPLC is entered at around 60 °C because increasing the temperature requires increasing the outlet pressure above the atmospheric pressure to prevent a phase transition of the mobile phase in the HPLC system. The upper temperature can then be defined as the point where every mobile phase which is used in reversed phase HPLC will turn into a supercritical fluid. While most of the organic solvents will become a supercritical fluid around 200 to 250 °C, the highest critical temperature is observed for water at 374 °C.

Now that the domain of high-temperature HPLC has been defined, which extends from 60 to 374 °C, the question of what is the useful temperature range needs to be addressed. Although in some fields of application, temperatures as high as 60 °C will not be tolerated and are considered too high to be used, the application of temperatures as high as 370 °C with a pure water mobile phase has been reported in the literature [8]. Even if the complete temperature range can be used for high-temperature HPLC, a limited temperature range is currently available for routine analysis. The reason is that the technical requirements dictate the real upper limit. Most conventional LC heating systems are only capable to raising the temperature to 80 °C. Although it is possible with every chromatographic system which is equipped with a column oven to enter the domain of high-temperature liquid chromatography, the region cannot be exploited further. Therefore, some instrument manufacturers have developed special heating systems which can generate temperatures as high as 200 °C [9-11]. The second aspect which has to be considered is the stability of the stationary phase. When HPLC was young, silica based reversed phase materials tended to rapidly degrade even at moderately elevated temperatures around 80 °C. It is therefore not surprising that alternative materials with an enhanced temperature stability like polystyrene-divinylbenzene (PS-DVB) or polymer-coated metal oxide stationary phases were already being examined at the end of the 1980s. Although silica-based phases have long lagged behind, they are now catching up in terms of stability at high temperatures. In some cases, they are even more stable than their metal oxide based counterparts. From many recent studies it can be deduced that temperatures as high as 200 °C will not lead to an immediate

collapse of the column and some columns can be used over a reasonably long time without total degradation [12, 13]. It can be summarized that although the domain of high-temperature liquid chromatography potentially extends up to 374 °C, the useful temperature range for routine analysis is currently limited to approximately 200 °C. This is quite reasonable because specially designed heating systems as well as suitable stationary phases both generating and withstanding these temperatures are now commercially available.

It is useful to have defined what can be understood of high-temperature HPLC, but now the question has to be addressed why is it beneficial to increase temperature?

First of all I would like to discuss the influence of temperature on efficiency and speed. This is based on the van-Deemter equation, which should be known to every chromatographer and can be written as:

$$H_u = A + \frac{B}{u} + C \cdot u \qquad \text{Equation 1}$$

Here, the height equivalent to a theoretical plate H_u (HETP) depends on three terms, which are the band broadening due to Eddy diffusion (A-term), longitudinal diffusion (B-term) and the resistance to mass transfer between and within the mobile and stationary phases (C-term) and the mobile phase flow rate u. While the A-term can be regarded not to depend on temperature, the remaining B and C-term are both temperature-dependent. This is because the B-term is directly proportional to the diffusion coefficient D_M, which is also a function of temperature.

$$B \propto D_M \qquad \text{Equation 2}$$

$$C \propto \frac{1}{D_M} \qquad \text{Equation 3}$$

From a purely theoretical standpoint, the goal is always to minimize band broadening and thus minimizing H by adjusting the flow rate of the mobile phase to the optimum linear velocity. At velocities higher and lower than the optimum linear velocity there is an increase of H. However, when the temperature is increased, the profile of this curve changes. At elevated temperatures, the minimum of the H_u-curve is shifted to higher linear velocities. In addition, there is a much flatter increase of H at flow rates higher than the optimum. This means that if a separation is carried out at a mobile phase flow rate which is much higher than the optimum flow rate, the loss of efficiency at higher temperatures is less pronounced than at lower temperatures. The net benefit of operating HPLC columns at higher temperatures therefore is that the operator has not to worry so much about the flow rate as long as it is higher than the optimum linear velocity. However, it needs to be stressed that there is no absolute increase in the efficiency, because it is not possible to lower the minimum of the van-Deemter curve [14].

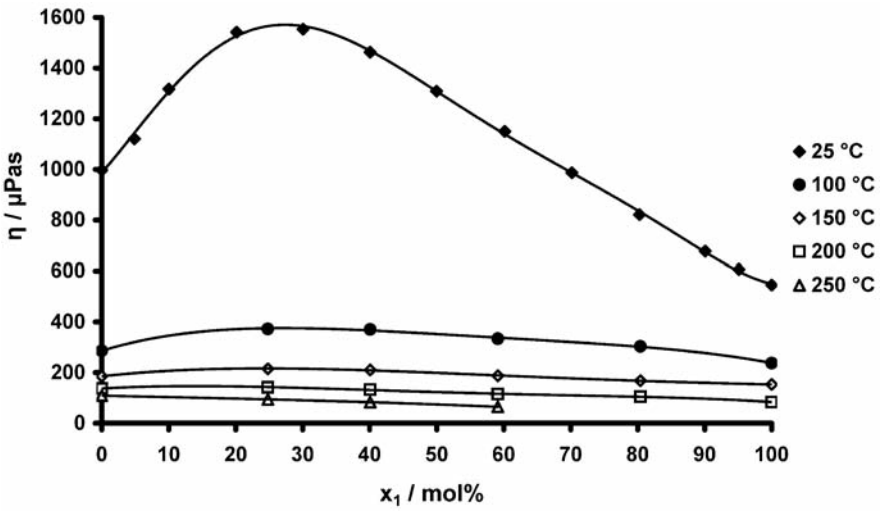

Figure 1. Viscosities of the binary mixture methanol (1) – water (2) at different temperatures. Additional data can be found in reference [15].

Another advantage of increasing the temperature is that the viscosity maximum of the mobile phase can be significantly reduced [15].

When a solvent gradient is applied, often a huge pressure maximum is observed. Every practitioner will have noticed that mixtures consisting of water and methanol are much more troublesome than the corresponding mixtures of water and acetonitrile at ambient temperature. However, by steadily increasing the temperature, this pressure maximum can be totally avoided as is shown in Figure 1.

The decrease of the mobile phase's viscosity at higher temperatures is also linked with the possibility to significantly speed up a chromatographic separation. In order to evaluate the effect of temperature on the theoretical gain in speed, we have to take a closer look at the diffusion coefficient D_M of the solute in the mobile phase. According to Wilke and Chang, the diffusion coefficient can be written as:

$$D_M = 7.4 \times 10^{-8} \frac{\sqrt{\Psi_2 M_2}}{\eta V_1^{0.6}} T \qquad \text{Equation 4}$$

where T is the absolute temperature, M_2 is the molecular weight of the solvent, V^1 is the molar volume of the solute and η is the viscosity of the mobile phase. Ψ_2 is the association factor for the solvent, which is generally assumed to be 1 for non-polar solvents, 1.9 for methanol and 2.6 for water [16]. If it is assumed that the molar volume of the solute is not influenced by temperature, the diffusion coefficient is directly proportional to the temperature and inversely proportional to the viscosity of the mobile phase, which is also a function of temperature. Remember that for liquids, the viscosity will always decrease as the temperature is increased. It can now be derived that at least theoretically, a tremendous gain in speed might be achieved when the temperature is increased up to 250 °C. The highest "speed" factors result if a solvent system consisting of water and isopropanol is considered. Then – at least theoretically – it would be possible to decrease the time for a separation which is carried out at 25 °C by the factor of 50 when the separation is carried out at 250 °C [15]. So while there is no absolute increase in the efficiency when the temperature is increased, temperature can be used to dramatically increase the speed of a separation.

The question remains how temperature can be incorporated into method development?

In principal, the effect of temperature on retention can be described by the van't Hoff equation, which can be written as:

$$\ln k = -\frac{\Delta H}{R} \cdot \frac{1}{T} + \frac{\Delta S}{R} + \ln \beta \qquad \text{Equation 5}$$

Here, ΔH is the enthalpy of transfer of the solute from the mobile into the stationary phase, ΔS is the entropy of transfer of the solute from the mobile into the stationary phase, R is the ideal gas constant and β is the volume phase ratio of the stationary and mobile phase. At least theoretically, for most analytes a plot of the natural logarithm against the inverse absolute temperature (ln k

versus 1/T) yields a straight line. In reversed phase HPLC, as the temperature is increased, retention will usually decrease [2,14]. Since the selectivity of the separation also depends on retention as is shown in Equation 6, temperature can be actively used to change the selectivity of the phase system and hence optimize resolution.

$$\alpha = \frac{k_2}{k_1}$$ Equation 6

A very nice example to demonstrate the potential of optimising resolution and analysis time has been given by Giegold et al. [1]. In Figure 2, an optimized separation of a mixture containing sulfonamides and trimethoprim is shown using a simultaneous temperature and solvent gradient. Neither the isothermal separation at 70 °C nor the isothermal separation at 90 °C were successful to achieve both a fast analysis time and a high resolution (data not shown here). In the case of the lower temperature, the last peak pair was not fully resolved while at the higher temperature, a co-elution of the first peaks was observed. In order to optimize the separation, a temperature gradient from 70 to 90 °C was performed simultaneously to solvent gradient programming. This example clearly shows that temperature is a powerful tool to adjust the selectivity and can be complementary to solvent gradient programming. Although this optimization was done by trial and error, work is currently carried out in the author's own laboratory to implement temperature programming in commercially available software designed for structured method development. With this tool, which will be incorporated into the DryLab® software (Molnar Institut, Berlin), the user will be able to use temperature programming in reversed phase HPLC to optimize chromatographic methods.

Although it was stated 30 years ago that temperature programming should yield the same results as solvent programming, solvent gradient elution is by far the most often used mode for method optimisation in HPLC. However, there are some special hyphenation techniques which could play an ever greater role in the near future. These comprise isotope ratio monitoring mass spectrometry (IRMS) [3,17] or the direct gustatory evaluation of separated species by a human being known as LC taste® [18]. This system uses the advantage of separation based on high-temperature HPLC and combines it with an in vivo detection of taste active compounds by a sensory tester or sensory panel. While for IRMS any addition of carbon to the mobile phase is strictly forbidden, LC taste® allows the use of mobile phase additives which are non-toxic and can be swallowed by a human being. Both techniques heavily depend on temperature programming. Here, the effect of decreasing the polarity of the mobile phase by increasing the temperature is exploited [7]. Hence, temperature programming is not only a concomitant tool to influence the selectivity of a separation, but it is detrimental if solvent programming cannot be used.

A final comment should be made to the heating system and the instrumental requirements. As was also outlined above, the heating system should be able to generate temperatures as high as 200 °C. A very important prerequisite to use high eluent temperatures is that the mobile phase can be adequately preheated to the temperature of the column. Otherwise, a severe band broadening or even peak splitting will be observed, leading to a complete loss of efficiency at higher temperatures. Meanwhile, most ovens are equipped with a device for eluent preheating and thus, the efficiency can be

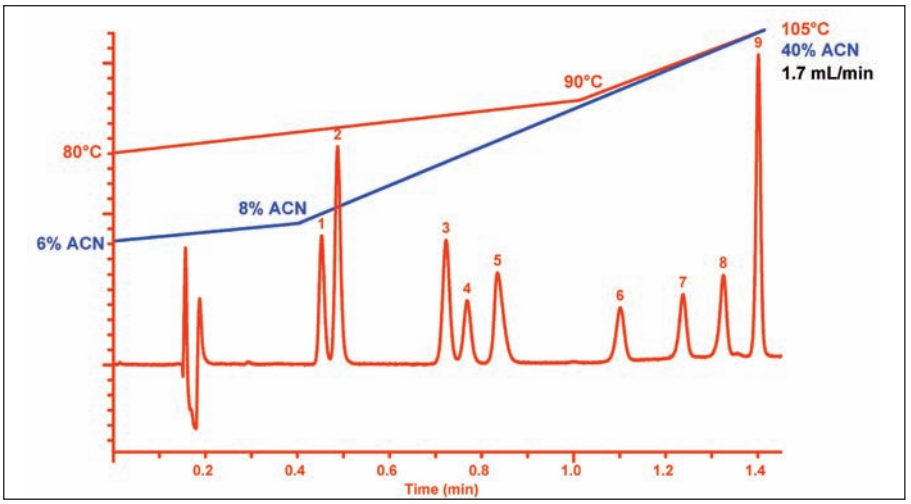

Figure 2. Chromatogram of the separation of a sulfonamide mixture and trimethoprim using a simultaneous solvent and temperature gradient on a Zorbax StableBond C-18 column. For experimental information, see reference [1].

Figure 3. Modular oven for high-temperature HPLC based on contact heating.

maintained. However, additional requirements must be met if the oven is used in temperature programmed mode. While air-based ovens might be used successfully for isothermal separations, block heating ovens have a much better heat transfer [19]. In some cases it might be necessary to apply fast temperature ramps of about 20 to 30 °C/min which cannot be achieved with air-based heating systems. An oven which is based on contact heating has recently been introduced to the market and offers the full capability of temperature programming, also including a fast cool-down of the mobile phase after a temperature programme (HT-HPLC 200, SIM Scientific Instruments Manufacturer, see Figure 3). The system is modular and allows for an independent adjustment of the preheating and the column temperature as well as a post column cooling of the eluent. This guarantees that the sensitivity is not compromised if a detector is used which is very sensitive to changes of the eluent's temperature.

A last word should be addressed to the HPLC system. In principal, any HPLC system can be used for high-temperature operation. The only modification is that a back-pressure regulator should be installed behind the column to keep the mobile phase in the liquid state. The highest vapour pressure is observed for the water-methanol system and is around 40 bar when pure methanol is used at 200 °C [20].

Conclusion

It can be concluded that temperature has a huge influence on all chromatographic parameters which are used to optimize a

method. This includes the retention of solutes, the selectivity of the phase system, the resolution of critical peak pairs as well as the polarity of the mobile phase. Temperature programming can be used to replace solvent programming when the composition of the mobile phase cannot be changed. The requirements for high-temperature HPLC are a heating system which should be modular and allows for precise and rapid heating of the mobile and stationary phases. What needs to be considered is the stability of the stationary phase. Besides metal oxide phases which are commercially available for nearly 20 years, the new generation of hybrid materials based on silica has been proven to be extremely stable at temperatures above 100 °C.

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