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Exploring the Use of Elevated Temperature for UPLC Applications

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Introduction

The use of elevated temperature in liquid chromatography has recently been an active area of research (1-4). As the temperature of the mobile phase is increased, several variables that play a key role in chromatographic performance are affected. Mobile phase viscosity is decreased at higher temperatures. This means that with a constant mobile phase, flow rate, column dimension and particle size, operating pressure is reduced at higher temperature. Analyte diffusivity is increased at higher temperature. This fact results in a shift in the optimum flow rate to higher values. Horvath (5) has discussed the fact that under conditions where kinetics are fast, efficiency is independent of temperature, and this fact has been verified by Lee et al (3) and Sandra et al (4). Therefore, at least theoretically, we should not expect higher temperature to provide increased plate count or resolution for a given column length and particle size. Instead, due to the flattening of the van Deemter curve and the reduced mobile phase viscosity, higher temperature will allow faster separations to be obtained with equivalent resolution. However, the use of longer columns to provide increased plate count and resolution is enabled by higher temperature (4). While these longer columns can be used at ambient temperatures, the flow rates achievable under these conditions would lead to very long separation times. It is important to point out that for these longer columns the optimum flow rate is in most cases not achievable even at higher temperature, since with increasing temperature the optimum flow rate also increases.

Conclusion

- High temperature LC and UPLC allow faster separations, selectivity benefits, and sharper peaks.
- Efficiency is **not** improved at higher temperatures. It only allows longer columns which only then would you achieve greater N
- Increasing column temperature requires higher flow rates in order to run within the optimal conditions.
- Analyte degradation is possible and must be examined in every case
- Narrow peaks require fast scanning UV and MS detectors, but not necessarily above 40Hz.
- Alternative solvents can be used at higher temperatures for unique selectivity

References

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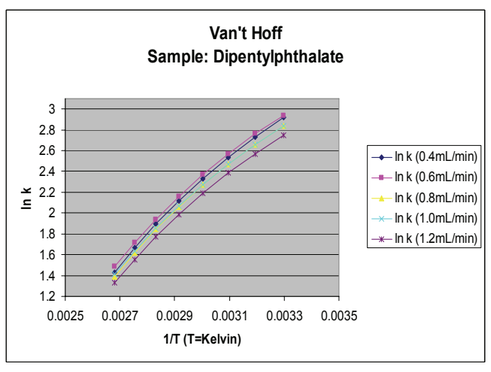
2. Y.Xiang, Y.Lui and M.Lee, *J.Chromatogr*, 1104 (2006) 198-202
3. Y. Xiang, B. Yan, B. Yue, C. McNeff, P. Carr and M. Lee, J.Chromatogr, 983 (2003) 83-89
4. F. Lestremau, A. Cooper, R. Szucs, F. David and P. Sandra, J.Chromatogr, 1109 (2006)191-196
5. F. D. Antia and C. Horvath, J.Chromatogr, 435 (1988) 1-15
6. Temperature and Pressure in Modern Chromatography Part 1: Theoretical Considerations Uwe D. Neue

Exploring Key Chromatographic Performance Aspects

Evaluation of Instrumentation Efficacy Through van't Hoff Plots

The experimental design must take into account the dependency of retention time due from temperature changes. Arrhenius equations are formulas used to determine the temperature dependence of a chemical reaction rate. When a reaction has a rate constant which obeys the Arrhenius equation, a plot of ln(*k'*)versus T^{-1} gives a straight line. These plots are more commonly known as van't Hoff plots. When applied to LC, we can measure the capacity factor (k') at varied temperatures and plot them versus the 1/T (where T = absolute temperature K). If we also take these measurements at various flow rates, the resulting curves should be linear. The figure below is a plot of ln(k') versus T^{-1} for dipentylphthalate at various flow rates and temperatures. The experiments were performed on an ACQUITY BEH C18 2.1 x 50mm, 1.7um column isocratically 40:60 with 0.1% formic acid and 0.1% formic acid in acetonitrile respectively.



	0.4 mL/min	0.6 mL/min	0.8 mL/min	1.0 mL/min	1.2 mL/min
Temp Δ	% Δ	% Δ	% Δ	% Δ	% Δ
30 to 40	16.84	16.15	16.26	16.49	16.32
40 to 50	17.98	17.44	17.25	16.87	16.67
50 to 60	18.43	18.29	18.06	17.84	17.70
60 to 70	19.20	19.01	19.05	18.62	18.48
70 to 80	19.81	19.77	19.59	19.22	18.82
80 to 90	20.81	20.17	20.09	19.72	19.80
90 to 100	20.98	20.40	20.79	20.08	20.04

Table 1. As a general rule of thumb, an increase in column temperature by 5°C will usually decrease retention(k) by 5-10%. Therefore, an increase of temperature by 10°C will decrease retention by 10-20%.

Effect of Temperature on Chromatographic Performance

Isocratic separations were done at constant percent organic and plots of plate count vs. flow rate were generated at several flow rates. Figure 3 shows the combined plot for the XBridge and ACQUITY columns at the two temperatures. Note that at 60°C the flow rate max was 750 mL/min, while at 90°C it was 1000 mL/min. The results for the XBridge column demonstrate that the highest plate count achieved is independent of temperature, about 12,000 plates. The same curves generated with the ACQUITY column show slightly different behavior. In this case, as the temperature is raised, the maximum achievable plate count also increases slightly, going from 22,000 at 60°C to 23,000 at 90°C. An interesting thing to point out about both graphs is that at the lower temperature, the plate count is higher at low flow rates, but at some point the plate count is equal or higher at the higher temperature.

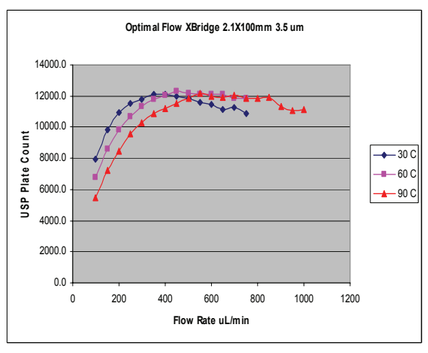


Figure 2. From the shown data we can conclude that as the column temperature is increased from 30°C to 90°C there is no increase in column performance but the optimal velocity has increased from 400uL/min to 650uL/min on a 2.1mm ID column.

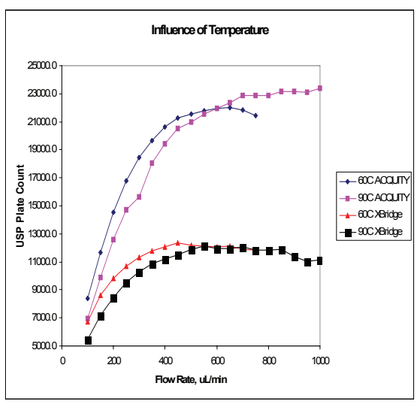


Figure 3. When the C term begins to dominate, the higher temperature provides increased plate count vs. lower temperature.

Effect of High Temperature on Detector Noise

There are concerns that using high temperature increases the noise observed on their detector, hence a post-cooling mechanism after the column is used on various instrumentation to cool the mobile phase prior to entry in the detector. Figure 4 (right) is an overlay comparison of the baseline of an isocratic lansoprazole experiment on the ACQUITY PDA. The extracted wavelength was 283.0nm. A comparison of the average detector noise within a one minute range of baseline was measured for the 40°C and 90°C. The resulting values for the 40°C and 90°C were 0.000013 AU and 0.000012 AU, respectively.

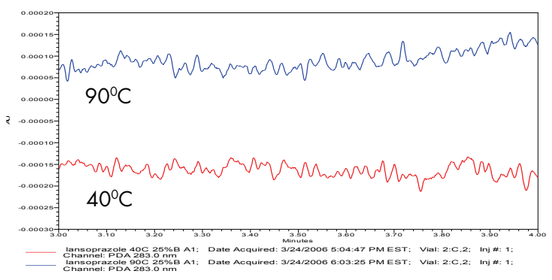
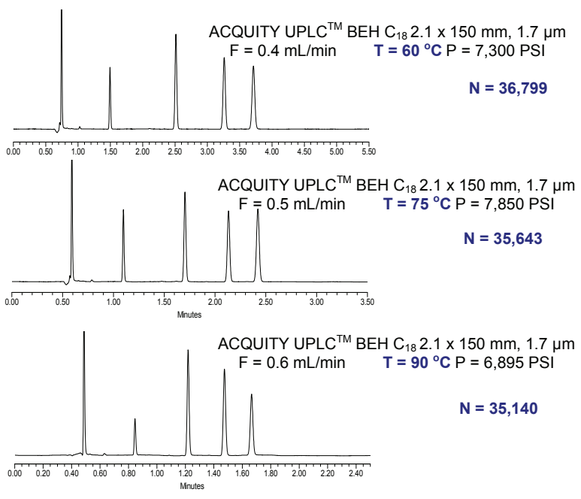


Figure 4. Baseline noise of ACQUITY PDA at high/low temp.

Typical Chromatographic Phenomena Related to Using Elevated Temperatures : *Predictable and Unpredictable*

Elevated Temperature = Speed Benefits ≠ Increased Efficiency

Isocratic



Gradient

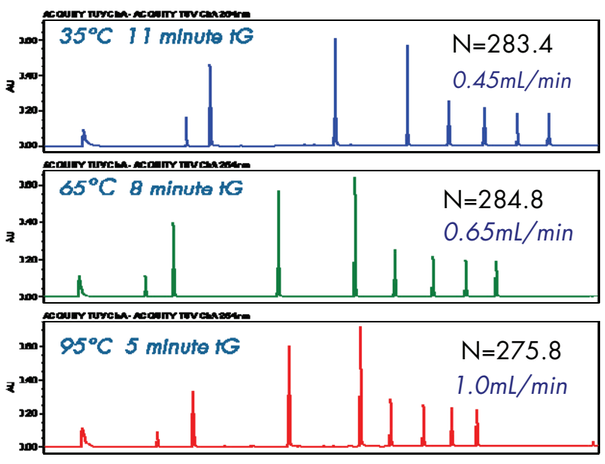


Figure 5 - 6: Figure 5 demonstrates the isocratic separation of several alkyl phenones on a 2.1x150 mm ACQUITY UPLC BEH C18, 1.7 mm column. The mobile phase temperature was varied from 60 to 75 to 90°C, while the flow rate was varied to provide a roughly constant back pressure of approximately 7-8K and therefore constant efficiency (6). The resulting efficiency stayed constant at about 35,000 plates. At 60°C, the flow rate was 0.4 mL/min, resulting in a run time of about 4 minutes. At 75°C, the flow rate was 0.5 mL/min, resulting in a run time of about 2.6 minutes. At 90°C, the flow rate was 0.6 mL/min, resulting in a run time of about 1.8 minutes. This example demonstrates that for this sample mix, running at 90°C vs. 60°C results in a two-fold reduction in analysis time, with no loss in resolution. The data shown in the figure 6 shows that as the column temperature is increased, the gradient time can be reduced and the peak capacity retained, Pc 30°C = 283, 60°C = 284 and 90°C = 275.

Effect of Temperature on Selectivity

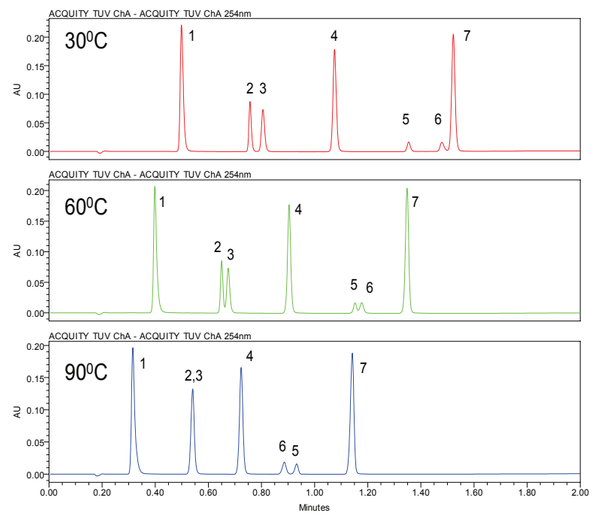


Figure 7: As the column temperature is increased the rate of mass transfer and solubility of the analytes changes differentially. This can result in changes in the order of elution and the resolution between the peaks. This is of particular interest when transferring methodology from analytical to preparative scale. We can see that the temperature can be used as a tool to manipulate the peak separation profile such as changing the organic modifier to affect selectivity. Thus temperature can be viewed as a tool rather than a necessity.

Thermally Labile Compounds

The drug products are desired to have shelf life longevity with inexpensive storage conditions. Experiments show degradation of Lansoprazole on-column at temperatures excess of 90°C with significant breakdown above 100°C.

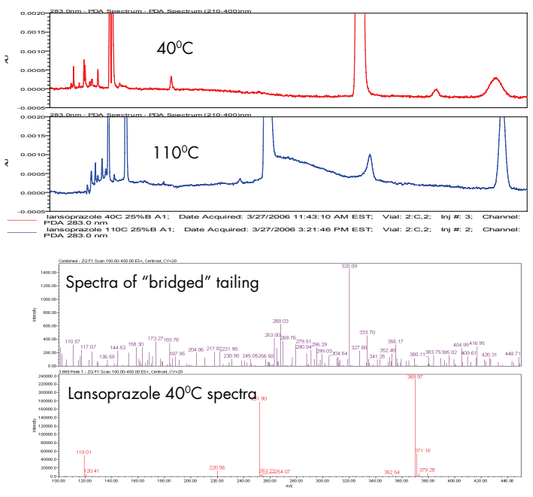


Figure 8: The combined extracted spectrum of the observed "bridged" phenomena tailing after the Lansoprazole analyte in the "blue" chromatogram is a result of on-column degradation yielding an m/z of 320 amu, a loss of ~48 amu.

Effect of Narrow Peaks on MS Data Quality

The combination of higher flow rates and longer columns gives rise to very narrow peaks approximately on average 1second width peaks which places a significant strain on the detection system to collect data at a sufficiently fast rate to define the peak without effecting the sensitivity or accuracy of the mass spectrometry measurement. The spectra obtained from a rat urine analysis (figure 9) yield the endogenous metabolites xanthuric acid (figure 10), hippuric acid (figure 11), kyurenic acid (2.3ppm), and pantothenic acid (1.4ppm) present in urine. We can see that analytes were collected with a mass accuracy of 3ppm or better.

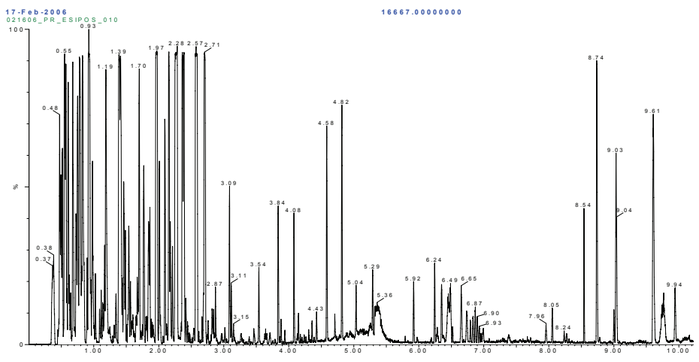


Figure 9: Metabolite ID in Rat Urine. ACQUITY UPLC BEH C18 2.1 x 150mm;1.7um. 90°C at 900uL/min. Peak capacity = 720. Peak widths ranged from just under 1sec to 3sec.

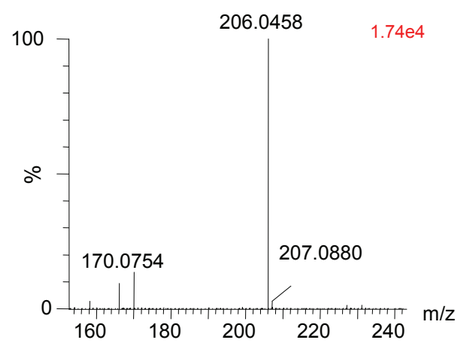


Figure 10: Xanthuric Acid Mass Accuracy = 2.4ppm

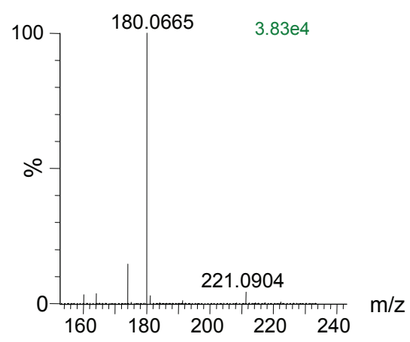


Figure 11: Hippuric Acid. Mass Accuracy = 2.8ppm

Enabling Viscous Solvents with Higher Temperature

Solvents such as isopropyl alcohol and dimethyl-sulphoxide (DMSO) have been used rarely by chromatographers due to viscosity of these solvents. Raising the column temperature to 90°C significantly reduces the viscosity. The data shown below (figure 12) illustrates how the back pressure of a typical 2.1 x 100mm C18 ACQUITY UPLC column changes over the period of a 0-100% organo-aqueous gradient, for acetonitrile, methanol and isopropanol. We can see from this data that the isopropanol solvent reaches a back pressure maximum of 11,000psi. The benefits of using mobile phase modifiers such as isopropanol (IPA) are three fold; 1) faster analysis, 2) sharper peaks and 3) different analyte selectivity

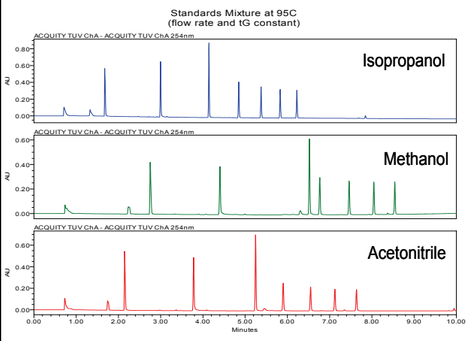


Figure 13: IPA offers speed. Compared to methanol and acetonitrile IPA gives a significant reduction in analysis time, this is because it has a greater elutropic strength.

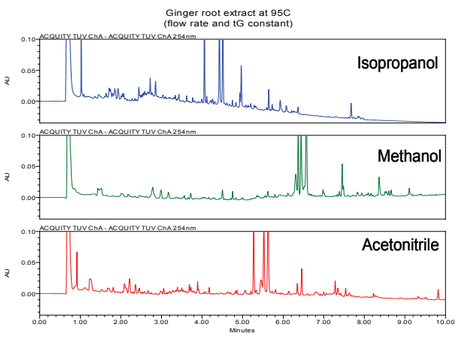


Figure 14: The analysis of a more complex sample, in this case ginger root extract illustrates the benefits of using IPA as an organic modifier. We can see that these are sharper and better resolved in the IPA separation

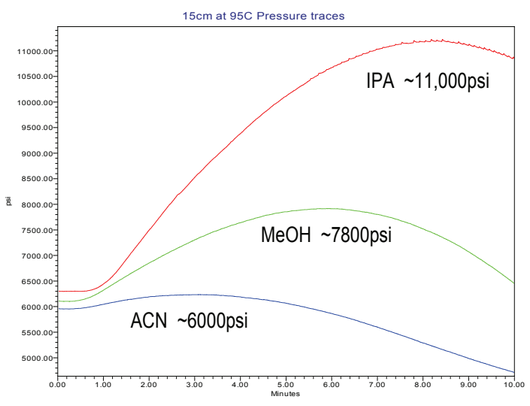


Figure 12: Organic modifier pressure profile at 95°C

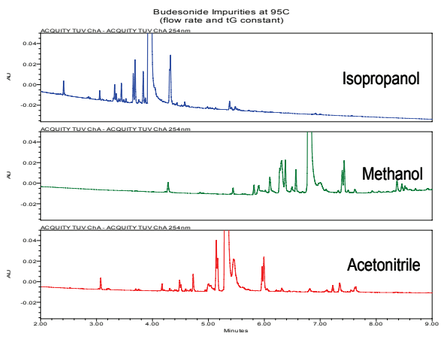


Figure 15: The selectivity advantages of IPA. The analysis of the corticosteroid budesonide. The order of elution of the peaks has changed with IPA, the two large impurity peaks that eluted after the budesonide peak elute in the methanol and acetonitrile elute before the budesonide peak with the IPA gradient

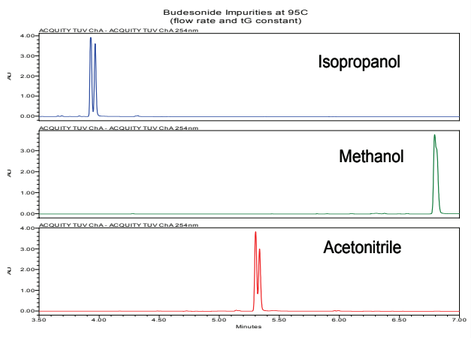


Figure 16: The two specific epimer peaks of budesonide are virtually baseline resolved with IPA , where as with methanol they are completely unresolved, despite being better retained, and are only approximately 40% baseline resolved with acetonitrile.

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