

High temperature to increase throughput in liquid chromatography and liquid chromatography-mass spectrometry with a porous graphitic carbon stationary phase

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Introduction

In recent years the use of high temperatures and temperature programming in liquid chromatography (LC) is becoming more popular in laboratories where high throughput is important. Mobile phase viscosity is reduced as separation temperature increases and therefore high flow rates can be utilised to achieve fast separations, without exceeding the standard operational pressure limits of the HPLC system. An additional benefit of using high temperature is that the lower mobile phase viscosity enhances the mass-transfer of the solute between the mobile and stationary phase, resulting in better chromatographic performance.

High separation temperatures in LC and liquid chromatography-mass spectrometry (LC-MS) with a temperature stable porous graphitic carbon (PGC) column are investigated in this work. Separation temperature was varied up to 200 °C, and the effect on retention, analysis time and sensitivity was measured. Analysis times were reduced by more than six-fold, whilst baseline resolution was maintained. The impact of the separation temperature on signal-to-noise ratio with atmospheric pressure chemical ionization (APCI) and electrospray (ESI) mass spectrometric detection was also investigated.

Methods

• Columns – Hypercarb™ 5 µm, 100 x 4.6 mm; Hypercarb 5 µm, 50 x 2.1 mm (Thermo Scientific, Bellefonte, PA)

• Instrumentation used to perform the UV work: HPLC system (quaternary pump with degasser, autosampler and variable wavelength UV detector) fitted with a programmable oven, Polaratherm™ Series 9000. The oven was operated with a temperature gradient or isothermally; the effluent cooler was set to 28° C.

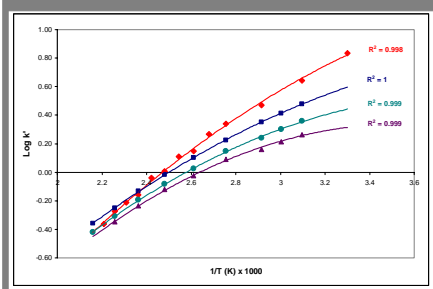
• Instrumentation used to perform the LC/MS work: Finnigan™ Surveyor™ MSQ™ (Thermo Scientific, San Jose, CA), fitted with Polaratherm Series 9000. In this system setup the effluent cooler was bypassed.

• To prevent evaporation of the mobile phase in the column at high temperatures, extra back pressure was introduced in the system, downstream from the column, by using 5 cm of 50 µm ID tubing.

Results

1. Effect of temperature on retention on porous graphitic carbon

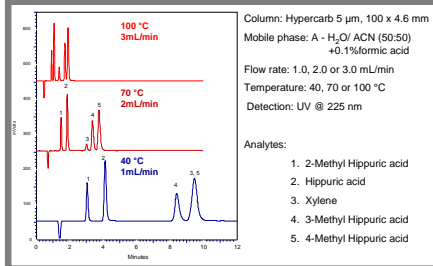
FIGURE 1. Effect of temperature (1/T) on retention (log k) for (◆) uracil, (■) phenol, (●) resorcinol, (▲) phloroglucinol. Temperature was varied between 30 and 190 °C. Regression coefficients (R²) are for a quadratic fitting.



The effect of varying separation temperature on the retention factors of several solutes is demonstrated in Figure 1 with a plot of log k versus 1/T (Van't Hoff plot [1]), for the temperature range between 30 and 190 °C. A quadratic relationship provides the best fit of the experimental data, with correlation coefficients R² > 0.998. This non-classical curvilinear Van't Hoff relationship has been attributed to a change of heat capacity of the system dependent on temperature [2], and is likely to be consistent with a change in the retention mechanism as temperature is increased.

2. High temperature to increase speed of analysis

FIGURE 2. Separation of xylene and hippuric acid positional isomers. Effect of temperature and flow rate on analysis time.



Xylene is metabolized in the human body and excreted as isomers of hippuric acid. The ability of Hypercarb to separate positional isomers is combined here (Figure 2) with elevated temperature LC to perform the analysis of xylene, hippuric acid and three methyl hippuric acid isomers in under 2 minutes (top chromatogram). The use of a separation temperature of 70 or 100 °C improves the separation by changing the elution order of xylene.

FIGURE 3. Separation of herbicides and metabolites at constant temperature (40 °C) or with a temperature gradient. USP resolution between peaks pairs 3,2 and 6,5.

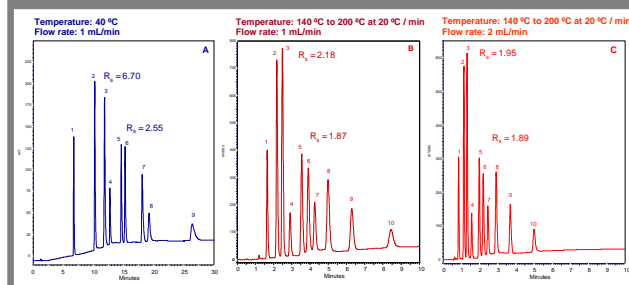
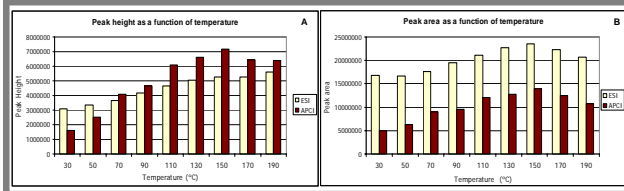


Figure 3 illustrates the gain in speed of analysis that can be obtained by using temperature programming. Seven herbicides and three metabolites of atrazine were separated with a solvent gradient of water and acetonitrile, at conventional temperature (Figure 3A). These compounds have a wide range of hydrophobicity, log P = 0.32 for atrazine-desethyl-1-desisopropyl and log P = 3.07 for propanil. Porous graphitic carbon [3] allows for good retention of the polar metabolites but also strongly retains hydrophobic analytes such as propanil. Under these mobile phase and temperature conditions propanil does not elute in 45 minutes; a stronger solvent such as isopropanol or tetrahydrofuran [4] instead would be required to elute this solute. In Figure 3B, the solvent gradient (5 to 100 % organic) is replaced with a temperature gradient from 140 to 200 °C at 20 °C/min and an isocratic mobile phase (50:50, water/acetonitrile); analysis time is reduced from 28 to 9 minutes, with full baseline resolution of all 10 analytes. Because at high temperatures the flow rate for optimum performance is higher [5], on Figure 3C the flow rate was increased to 2 mL/min (all other conditions kept unchanged), which further reduced analysis time to just over 5 minutes. The observed resolution between any pair of solutes is > 1.5 in all chromatograms.

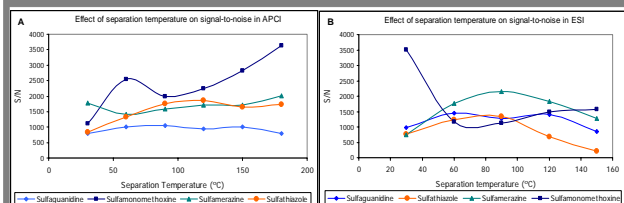
3. Effect of separation temperature on ESI and APCI sensitivity

FIGURE 4. Effect of the temperature of the mobile phase on the ESI and APCI signal, measured as peak height (a) and peak area (b) of sulfamerazine injected into the LC flow (no column). Mobile phase: H₂O/ACN (1:1) + 0.1 % formic acid for ESI, H₂O/ACN (1:1) for APCI; Flow rate: 0.2 mL/min for ESI, 0.5 mL/min for APCI.



To measure the changes in ionization efficiency with increased temperatures, the mobile phase temperature was varied between 30 and 190 °C and a basic compound (sulfamerazine) was injected into the LC flow. The detector signal was measured as peak height and peak area of the extracted mass chromatograms for [M+H]⁺ (m/z 265) and plotted as a function of the mobile phase temperature (Figure 4A and 4B respectively). It was observed that at higher temperatures there is an improvement in the signal intensity for both ESI and APCI, as can be seen on Figure 4B; peak area increases as the temperature of the mobile phase increases, with an optimum at approximately 150 °C. These improvements are believed to be a result of better analyte desolvation at higher temperatures and changes in solution chemistry in ESI or changes in gas phase chemistry in APCI, which enhance the ionization process. However, increased separation temperature also affects chemical noise, particularly in ESI, therefore the measure of S/N is a better probe of the sensitivity of the method in LC-MS. For this reason peak height was also measured and the observation (Figure 4A) is that there is a 4-fold increase in APCI and a 1.7-fold increase in ESI when the temperature of the mobile phase is increased from 30 to 150 °C.

FIGURE 5. Impact of the separation temperature on the signal-to-noise ratio (S/N) in APCI (A) and ESI (B)



Experimental conditions for APCI:

Column: Hypercarb 5 µm, 100 x 2.1 mm
Mobile Phase: Ammonium acetate 10 mM pH 9 / ACN (65:35)
Flow rate: 0.2 mL/min
Detection: +APCI

Experimental conditions for ESI:

Column: Hypercarb 5 µm, 50 x 2.1 mm
Mobile Phase: A - H₂O + 0.1 % formic acid, B - ACN + 0.1 % formic acid; Gradient: 50 to 100 % B in 5 min
Flow rate: 0.2 mL/min; Detection: +ESI

The effect of separation temperature on the S/N of four sulfonamides was measured in LC-APCI-MS and LC-ESI-MS (Figures 5A and 5B respectively). The change in peak height has a chromatographic contribution, and a detection contribution, as discussed above. As the column temperature increases, retention is reduced and therefore the chromatographic peak becomes narrower and taller. The detection contribution is demonstrated in Figure 4.

In APCI the separation temperature for the best S/N is 180 °C, except for sulfaguanidine which exhibits an optimum at 90 °C and sulfathiazole that exhibits an optimum at 120 °C. In ESI however, above separation temperatures of 90 to 120 °C, noise increases more rapidly than the signal intensity, therefore, S/N drops as temperature is increased. The S/N curve profile for sulfamonomethoxine is different from the other sulfonamides; at 30 °C this solute is strongly retained and elutes with 100 % organic mobile phase. However, when the temperature is raised it elutes with a lower percentage of organic, therefore, there are three variables determining the signal intensity: retention that affects peak width, separation temperature (temperature of the mobile phase) and percentage of organic in the mobile phase.

Conclusions

- It was observed that increased column temperatures result in decreased retention times; the relationship between capacity factor (log k) and absolute temperature (1/T) is quadratic between 30 and 180 °C.
- By using high separation temperatures or temperature gradients, up to six-fold gains in speed of analysis were observed.
- The signal-to-noise ratios in LC-MS were measured at temperatures between 30 and 180 °C and improvements of 1.7-fold for ESI at 90 °C and 4-fold for APCI at 180 °C were observed. Temperatures higher than 90 °C produce an increase in ESI signal but also in noise, which may actually decrease S/N.

References

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Additional Information

For additional information, please browse our Chromatography Resource Centre which can be accessed from: www.thermo.com/columns.

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