

# Advantages of Using Ultra High Temperature in High Throughput LC and LC/MS

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## Overview

The use of very high temperatures (up to 200 °C) in liquid chromatography has advantages in terms of throughput, sensitivity of the analysis and peak capacity. The work presented in this poster demonstrates gains of up to 7 fold in signal-to-noise ratio with APCI, and reduction in analysis time of 5 to 8 fold.

## Introduction

Ultra High Temperature Liquid Chromatography (UHT-LC) operates at temperatures up to 200 °C. Temperature affects the equilibrium distribution of the sample between the mobile phase and the stationary phase, measured as retention factor (K) and, to some extent, it may also affect selectivity (α). The use of high temperatures in reversed-phase liquid chromatography has several advantages:

- Higher peak capacity** – at higher temperatures solubility is reduced which enhances the mass-transfer between mobile and stationary phases resulting in higher efficiencies, providing sharper peaks and increased peak capacity
- Higher sensitivity** – at high temperatures peaks are more efficient and sharper, thus there is an improvement in peak height, and better signal-to-noise ratios are obtained.
- Higher speed** – at high temperature backpressure is reduced allowing for higher flow rates to be utilized for fast separations without compromising efficiency. According to the van Deemter equation, the increase in plate height (H) with flow rate (above optimum) is slower at higher temperatures.

UHT-LC has advantages when coupled with mass spectrometry. In LC/MS with electrospray (ESI) the column effluent is nebulized in the ion source by a high voltage applied to the electrospray needle and a nebulizing gas, followed by droplet desolvation with high temperature. In atmospheric pressure chemical ionization (APCI), the column effluent is vaporized by a high temperature applied to the probe and a nebulizing gas, followed by ionization in the gas phase. When UHT-LC is used in combination with ESI and APCI, the mobile phase reaches the ion source at elevated temperature which aides the vaporization and desolvation processes, thus increasing the ionization efficiency and consequently the sensitivity of the analysis.

UHT-LC has specific requirements in terms of column stability. Columns packed with alkyl-modified silicas, which are generally used in RP-LC, should not be used above 60 – 80 °C (this limit is dependent upon the silica, ligand and mobile phase pH). At these extreme temperatures, hydrolysis of the organosilane bond or dissolution of the silica may occur. Porous graphitic carbon (Hypercarb™, summary of properties in Table 1) is the ideal stationary phase for UHT-LC, since it is not affected by physical or chemical degradation at high temperature regardless of mobile phase used (temperatures up to 2500 °C are used in the manufacturing process). Hypercarb is 100% carbon, and thus chemically very stable and robust. It has been demonstrated<sup>1</sup> that Hypercarb is the only stationary phase available in the market that can be routinely used up to 200 °C under isothermal or temperature gradient conditions. Moreover, when used in UHT-LC/MS, there is no phase bleed.

Ovens with temperature programming, mobile phase preheating, and mobile phase pre-detector cooling are now commercially available. Mobile phase preheating is an important requirement at column temperatures above 80 °C to prevent band dispersion caused by thermal mismatch across the diameter of the column. When using UV detection it is also necessary to cool the mobile phase before it reaches the flow cell, to prevent damage.

TABLE 1. Chromatographic properties of Porous Graphitic Carbon.

Particles	Spherical, fully porous	Manufactured from silica template.
Particle size	3.5 and 7µm (30µm)	Ensures good packing bed uniformity.
Surface area	120 m <sup>2</sup> /g	Ensures retention linearity and good loading capacity.
Median pore diameter	250 Å	Ensures good mass transfer for wide range of analyte shapes and sizes.
% Carbon	100 A	Chemical stability; long lifetime; high temperature stability.

In the work presented in this poster the effect of column temperature on the capacity factor, the speed of analysis and on sensitivity (ESI and APCI signal intensity and signal-to-noise ratio) is demonstrated. Data on column stability is also presented.

## Methods

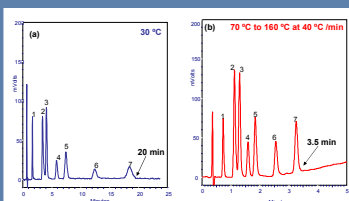
- Columns – Hypercarb 5 µm, 100 x 4.6 mm; Hypercarb 5 µm, 50 x 4.6 mm; Hypercarb 5 µm, 50 x 2.1 mm; Hypercarb 5 µm, 100 x 2.1 mm (Thermo Electron Corporation)
- Instrumentation used to perform the UV work: HPLC system (quaternary pump with degasser, autosampler and variable wavelength UV detector) fitted with a programmable oven, Polartherm Series 9000. The oven was operated with a temperature gradient or isothermally; the effluent cooler was set to 25° C.
- Instrumentation used to perform the LC/MS work: Finnigan™ Surveyor™ and Finnigan™ Surveyor™ MSQ™, fitted with Polartherm™ 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 backpressure was introduced in the system, downstream from the column, by using 50m of 50 µm tubing

## Results

### Faster analysis at high temperatures

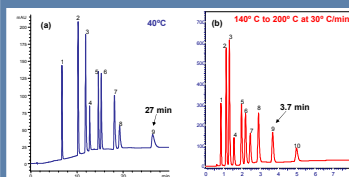
Figures 1 and 2 illustrate the gain in speed of analysis and peak capacity that can be obtained by using temperature as a method development parameter. In Figure 1 the isocratic separation of seven phenolic acids is reduced from 20 minutes to 3.5 minutes by running a temperature gradient from 70 to 160 °C. A further advantage of the temperature gradient is that, unlike an organic gradient, it allows for sensitive detection at low UV wavelength. In Figure 2, seven herbicides and three metabolites of atrazine are separated in 5 minutes with a temperature gradient. These compounds have a wide range of hydrophobicity (log P 0.32 for atrazine-desethyl-1-desisopropyl and log P 3.07 for propanil); at 40° C propanil does not elute in 45 minutes.

FIGURE 1. Separation of phenolic acids at 30°C (a) and with a temperature gradient (b). Analysis time is reduced from 20 to 3.5 minutes.



Column: Hypercarb, 5 µm, 50 x 4.6 mm  
Mobile Phase: A – H<sub>2</sub>O+0.1% TFA; B – ACN+0.1% TFA; (a) 15:85; (b) 20:80.  
Flow rate: (a) 1 mL/min; (b) 2 mL/min.  
Temperature: (a) 30 °C; (b) 70 °C to 160 °C at 40 °C/min.  
Detection: UV at 205 nm  
Analytes: 1. Benzoate; 2. Dicamba; 3. MCPP; 4. MCPA; 5. 2,4-D; 6. MCPB; 7. Benzoil

FIGURE 2. Separation of herbicides and metabolites at 40°C (a) and with temperature gradient (b). Over seven-fold reduction in analysis time.



Column: Hypercarb, 5 µm, 100 x 4.6 mm  
Mobile Phase: A – H<sub>2</sub>O; B – ACN; (a) Gradient: 5% to 100% B in 15 min; (b) isocratic (50:50).  
Flow rate: (a) 1 mL/min; (b) 2 mL/min.  
Temperature: (a) 40 °C; (b) 140 °C to 200 °C at 30 °C/min.  
Detection: UV at 215 nm  
Analytes: 1. Atrazine-desethyl-1-desisopropyl; 2. Atrazine-desethyl; 3. Atrazine-desisopropyl  
4. Propanil; 5. Prometryn; 6. Atrazine; 7. Ametryn; 8. Simazine; 9. Symetryn; 10. Propanil

## UHT-LC/MS

It has been demonstrated<sup>2</sup> that an increase in the column temperature benefits chromatographic peak height. The effect of the temperature of the mobile phase on the ionization efficiency in ESI and in APCI was investigated by flow injecting a basic compound (sulfamerazine) at temperatures ranging from 30 to 190 °C, and by measuring the signal intensity (plot of the signal vs temperature in Figure 3). As expected, at higher temperatures there is an improvement in the signal for both ESI and APCI. This effect is stronger in APCI because with this technique, vaporization occurs prior to ionization in the gas phase. When the preheated mobile phase reaches the ion source, vaporization is enhanced, thus ionization is more efficient. However, increased separation temperature also affects noise, particularly in ESI. Figure 4 shows the effect of separation temperature on the signal-to-noise ratio (S/N) of four sulfonamides measured in APCI and ESI. In APCI the separation temperature for best S/N is 180 °C, except for sulfaguanine which has its optimum at 90 °C. In ESI however, above 90 to 120 °C the increase in noise is greater than the increase in signal intensity, therefore S/N drops.

FIGURE 3. Effect of temperature of the mobile phase on the ESI and APCI signal of sulfamerazine.

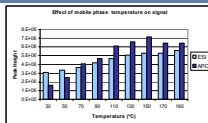


FIGURE 4. Impact of the separation temperature on the signal-to-noise ratio in APCI and ESI for sulfonamides.

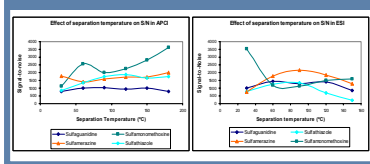
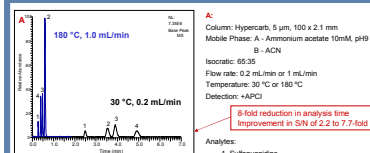
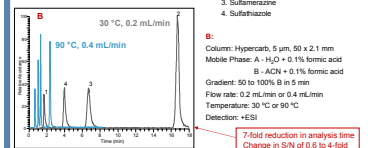


Figure 5 illustrates the gain in sensitivity and throughput when the separation temperature is increased. In APCI when the temperature is increased from 30 to 180 °C (Figure 5A), the S/N increases 2.2 to 7.7-fold depending on the compound, with a 8-fold reduction in analysis time.

FIGURE 5. LC/MS of sulfonamides: impact of the separation temperature on the analysis time and signal intensity: A – APCI; B – ESI. Base peak chromatograms normalized to highest signal.

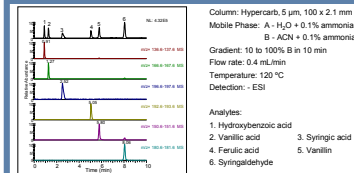


8-fold reduction in analysis time  
improvement in S/N of 2.2 to 7.7-fold



7-fold reduction in analysis time  
Change in S/N of 0.8 to 4-fold

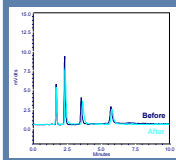
FIGURE 6. UHT-LC/ESI-MS of phenolic compounds



Column: Hypercarb, 5 µm, 100 x 2.1 mm  
Mobile Phase: A – H<sub>2</sub>O + 0.1% ammonia  
B – ACN + 0.1% ammonia  
Gradient: 10 to 100% B in 10 min  
Flow rate: 0.4 mL/min  
Temperature: 120 °C  
Detection: - ESI

- Analytes:
1. Hydroxybenzoic acid
  2. Vanillic acid
  3. Syringic acid
  4. Ferulic acid
  5. Vanillin
  6. Syringaldehyde

FIGURE 7. Hypercarb column stability under high temperature conditions.



Column stability and performance is an essential element of UHT-LC. Hypercarb column performance was monitored by running the QC test mixture when the column was new, and again after the column had been used under high temperature conditions and acidic mobile phases over a period of 6 weeks (Temperatures will have ranged from 100–200°C for extended periods). In Figure 7 the two chromatograms obtained are compared. Retention times are approximately the same and the efficiency of the last eluting peak (3,5-xylene) decreases by less than 10%.

## Conclusions

The work presented in this poster demonstrates the advantages of UHT-LC with UV and MS detection:

- Higher peak capacity – at high temperatures peaks are sharper, therefore, peak capacity is increased.
- Higher speed – reduction of 5 to 8 fold in run times was accomplished with high temperatures.
- Higher sensitivity – at high temperatures peaks are sharper, therefore, an improvement in peak height is obtained. Additionally, in LC/MS with ESI and APCI higher separation temperatures aid in desolvation and vaporization thus increasing ionization efficiency.

The ability to work at very high temperatures relies on column stability and performance. Hypercarb's stability and unique ability to retain polar compounds and to separate closely related molecules make this phase ideal for UHT-LC with any detection technique.

## References

- (1) S.J. Marin, B.A. Jones, W.D. Felix, J. Clark, J. of Chromatog., 1030 (2004) 255-262.
- (2) L. Pereira, S. Aspey, M. Woodruff, Poster presented at HPLC 2004, Philadelphia.

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