# Use of high temperature in LC to achieve high resolution separation of complex mixtures

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## Abstract

**Purpose:** To demonstrate the improvements in chromatographic resolution when column length is increased by coupling up to 5 columns and operating at high temperature (110 - 120 °C) to reduce system pressure.

## Introduction

The use of high temperatures in reversed–phase liquid chromatography has several practical advantages. Mobile phase viscosity is reduced at higher temperatures [1] which enhances the mass-transfer of the solute between the mobile and stationary phase, resulting in better chromatographic performance.

Another advantage of reduced solvent viscosity at high temperatures is reduced backpressure, allowing for the use of higher flow rates to increase speed of the analysis, without losing efficiency. In fact, optimum linear velocity increases proportionally to T/η (temperature / eluent viscosity) [2], and therefore the flow rate for optimum efficiency is shifted to a higher value at higher temperatures. As a consequence, the analysis speed can be improved by 5 to 15-fold, when temperature is increase from ambient to 200 °C. Reduced backpressure at high temperature also allows longer columns packed with small particles to be used to facilitate the resolution of complex samples.

The pressure drop across the column varies with the length and diameter of the column, the diameter of the particles packed in the column, the flow rate through the column and the viscosity of the mobile phase, as indicated by Equation 1.

*Equation 1.*

$$P \cong \frac{250\, L\, \eta\, F}{d_p^2\, d_c^2}$$

L - column length (cm), η - viscosity (cP), F - flow rate (mL/min)
$d_p$ – particle diameter (μm), $d_c$ – column internal diameter (cm)

Resolution ($R_s$) is proportional to the square root of separation efficiency (N), as described by Equation 2 which expresses resolution as a function of capacity factor (*k*), selectivity (α), and efficiency (N).

*Equation 2.*

$$R_s = \frac{1}{4}\,\frac{(\alpha - 1)}{\alpha}\,\sqrt{N}\,\frac{k}{1+k}$$

α - selectivity, N – efficiency, *k* – retention factor

The ability to work at very high temperatures relies on column stability and performance. Porous graphitic carbon (PGC) is a robust stationary phase for high temperature LC, since it is not affected by physical or chemical degradation at high temperature regardless of mobile phase used. It is 100% carbon, thus chemically very stable and robust, and it can be routinely used up to 200 °C under isothermal or temperature gradient conditions.

In the work presented in this poster the chromatographic resolution is maximised by coupling several porous graphitic carbon columns in series to a total column length of 500 mm. Experimental parameters evaluated are effective column length, separation temperature, flow rate and mobile phase composition.

## Materials & Methods

- Columns – Hypercarb™ 3 μm, 100 x 2.1 mm (Thermo Scientific, Bellefonte, PA), 5 columns. Columns were coupled using 5 cm of stainless steel tubing 0.005" ID.
- Instrumentation: Surveyor™ HPLC system (quaternary pump with degasser, autosampler and photodiode array UV detector) fitted with a programmable oven, Polaratherm™ Series 9000.The effluent cooler was set to 28 °C.
- Test method I (Figures 1, 2 and 3):

  Mobile phase: $H_2O$ + 0.1%formic acid / ACN + 0.1%formic acid (60:40)

  Flow rate: 0.2 mL/min; Detection: UV at 254 nm;

  Analytes: Figures 1 and 2: 1. Uracil; 2. Atrazine desethyl deisopropyl; 3. Hydroxybenzoic acid; 4. Vanillic acid; 5. Propazine; 6. Atrazine.
- Test method II (Figure 4):

  Mobile phase: $H_2O$ / ACN (90:10)

  Temperature: 110 °C ; Detection: UV at 254 nm

  Analytes: 1. Acetone; 2. Uracil; 3. Phenol; 4. Atrazine desethyl deisopropyl
- Conditions for Figure 5 (p-nonylphenol):

  Mobile phase: A - $H_2O$ + 0.1%formic acid; B - ACN + 0.1%formic acid

  Gradient: 50 to 70% B in 30 min (for 1 column); 25 to 70% B in 120 min (for 5 columns)

  Flow rate: 0.2 mL/min (for 1 column); 0.3 mL/min ( for 5 columns)

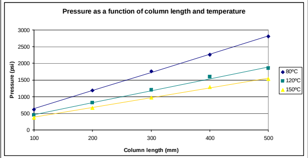
  Temperature: 40 °C (for 1 column); 110 °C ( for 5 columns)

  Detection: UV at 204 nm.

## Results

### i) Pressure

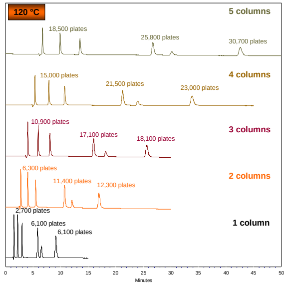
**FIGURE 1. Pressure as a function of the column length and temperature.**




When the separation temperature is increased the mobile phase viscosity is reduced, and as a result the pressure drop across the column is reduced. This enables a higher flow rate or a longer column to be used for an equivalent pressure drop. Figure 1 relates pressure with column length and temperature. At conventional temperatures (30 to 40 °C) it would not be possible to use more than 300 mm of column packed with analytical particle sizes.

### ii) Efficiency and resolution

**FIGURE 2. Increased efficiency with increasing column length. Separation temperature 120 °C.**




Because of the reduced pressure at high temperatures it was possible to couple 5 columns with a total length of 500 mm, when running the separation at 120 °C. Figure 2 shows the improvement in separation efficiency: over 30,000 effective plates where achieved with 500 mm of column, 5 times higher than obtained with the 100 mm single column.

Resolution is proportional to the square root of efficiency and therefore a 2.2 fold improvement in resolution is expected when increasing the separation efficiency by 5 fold. Table 1 details the resolution (USP) for each pair of peaks in Figure 2. The improvement in resolution is between 2.1 and 3 fold, in agreement with the expected value; this indicates that there is no significant loss in performance when the columns are coupled together.

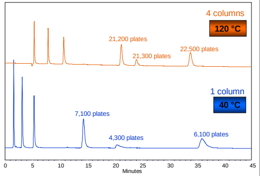
This gain in resolution is particularly important when dealing with the separation of complex samples.

**Table 1. Resolution (USP) as a function of the number of columns.**

| Peak pair | Number of columns (total length) | | | | |
|---|---|---|---|---|---|
| | 1 (100mm) | 2 (200mm) | 3 (300mm) | 4 (400mm) | 5 (500mm) |
| 2,1 | 4.07 | 6.60 | 8.96 | 10.78 | 12.21 |
| 3,2 | 4.28 | 6.41 | 8.28 | 9.66 | 10.66 |
| 4,3 | 11.43 | 16.32 | 20.39 | 22.94 | 25.34 |
| 5,4 | 2.15 | 3.24 | 4.20 | 4.45 | 4.98 |
| 6,5 | 6.70 | 9.20 | 11.29 | 12.76 | 14.39 |

### ii) Run time

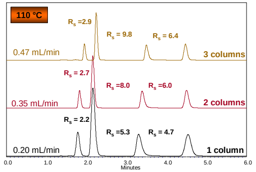
**Figure 3. Constant run time with 1 and 4 columns, by varying the separation temperature.**




The separation of the same test mixture at conventional temperature, 40 °C, on a 100 mm column takes approximately the same time as the separation on 4 columns at 120 °C. The advantage of working at high temperature and with a longer column is the improvement in separation efficiency.

The change in temperature from 40 to 120 °C also generates a change in selectivity. Changes in selectivity with temperature on porous graphitic carbon are reported in the literature [3].

**Figure 4. Constant run time with 1, 2 and 3 columns, by varying flow rate.**

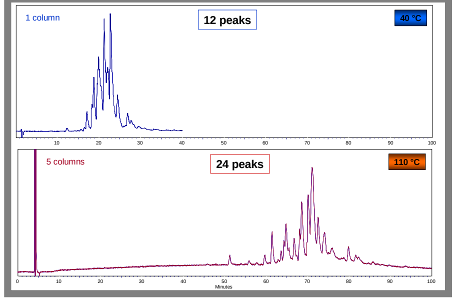



Increased efficiency is obtained at the expense of analysis time, for constant temperature and constant flow rate.However, the flow rate for optimum efficiency is shifted to a higher value at higher temperatures, and therefore, no loss of efficiency is observed by increasing the flow rate. In Figure 4 the flow rate was increased, as the column length (or number of coupled columns) increased to maintain the run time of 6 minutes. The advantage is that resolution is improved by at least 32%.

### iii) p-Nonylphenol sample

The 5-column system was used to separate p-nonylphenol, which is a mixture of several isomers due to the branching of the C-9 alkyl group. The chromatogram is compared with the one obtained with a single column run at 40 °C in Figure 5. The resolution is significantly improved and, as a result, the number of identifiable peaks doubles.

**FIGURE 5. Comparison of the separation of p-nonylphenol with a 5 column system at 110 °C and a single column at 40 °C.**




## Conclusions

- At high temperature column length can be increased to increase separation efficiency without exceeding the pressure limits of the system.
- By coupling 5 columns (total length 500 mm) over 30,000 effective plates were obtained and resolution was improved by 2.1 to 3 fold.
- On a 4-column system run at 120 °C the run time is similar to that on a single column run at 40 °C, but with improved efficiency.
- At high temperature flow rate can be increased to reduce analysis time when working with long columns, without exceeding the pressure limits of the system. Improvements of over 32% in resolution were observed.
- The gain in resolution obtained when using long columns at high temperatures is demonstrated for a complex mixture of p-nonylphenol.

## References

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(2) D. Guillarme, S. Heinisch, J.L. Rocca, *J. of Chromatogr. A* **2004**, 1052, 39-51.
(3) L. Pereira, S. Aspey, H. Ritchie, *J. Sep. Sci.*, in press

## Additional Information

For additional information, please browse our website: www.thermo.com/columns



PO20376_E02/07