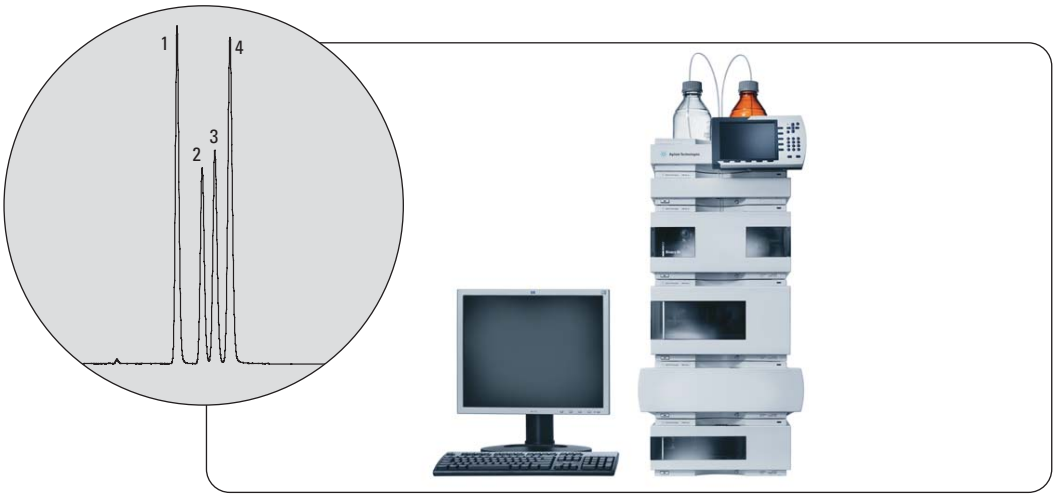
Using elevated temperatures with the Agilent 1200 Series Rapid Resolution LC system for more speed, more resolution, and better peak shape in LC applications

Technical Note



Introduction

A recent trend in LC analysis today is to increase sample throughput to achieve higher laboratory productivity and efficiency. One way to speed up LC analysis is to replace long columns with short ones, to increase the flow rate and gradient steepness, and to use LC systems that are capable of delivering gradient changes to the column with very low delay times. A parameter, which is not too often mentioned during these procedures, is the optimization of the column compartment temperature. A possible reason might be that LC systems, which are able to provide column compartment temperatures up to 100 °C and which also provide a cooling device in front of the detector, are not commercially available. Even though other authors have already proven the benefit of elevated temperatures for the analysis of small molecules, column temperatures above 60 °C are rarely used.
This Technical Note demonstrates that elevated temperatures up to 100 °C help to increase speed of analysis and also help to improve resolution and peak shape. The design of the Agilent 1200 Series column compartment SL is shown and it is demonstrated that cooling of the column effluent before the detector can significantly reduce noise, especially for high flow rates and elevated column temperatures.

Agilent Technologies

Equipment and materials

An Agilent 1200 Series Rapid Resolution LC system was used with the following modules:

- Agilent 1200 Series binary pump SL and vacuum degasser for high-speed and high-resolution applications on short and long sub 2-µm particle columns
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermo-statted column compartment SL with new design for column temperatures up to 100 °C
- Agilent 1200 Series diode-array detector SL for 80-Hz operation, including new data protection tool
- ZORBAX SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particles

Faster analysis time

An increase of column temperature reduces the viscosity of mobile phases and consequently the column backpressure decreases. Lower backpressures enable higher flow rates and faster analysis run times. Figure 1 is an example that shows to what extent the analysis time can be reduced using a column temperature of 95 °C. In conventional LC, columns packed with 5 or 3.5-µm particles perform as shown by the upper trace in figure 1. A column temperature of about 40 °C is frequently used. This resulted in analysis times of about 11 min whereby good resolution between peak 4 and 5 was obtained. The peak width at half height for the last peak was 3.4 s, which is a typical value for this type of analysis. Reducing the length and the particle size of the column shortened the analysis time and decreased the peak width for the last peak down to 0.5 s while maintaining resolution performance. Further shortening of the analysis time was achieved by increasing the temperature to 95 °C. The analysis time was shortened to 0.4 min while resolution still remained very good. The peak width for the last peak was down to 0.197 s. The gain in speed was increased up to a factor of 27.

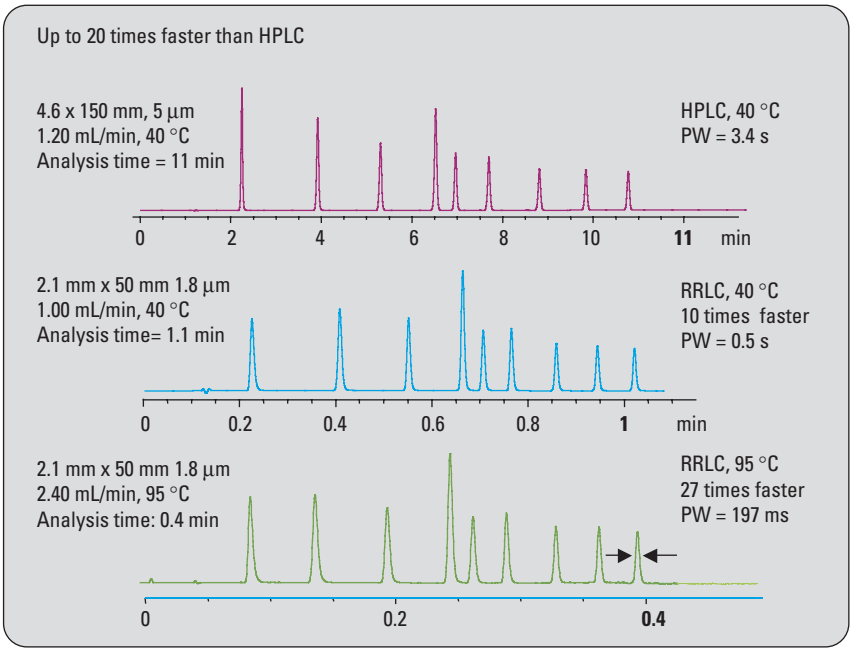


Figure 1
Influence of elevated column temperature on analysis speed.

Chromatographic conditions

Sample:	"Phenone-Mix" (0.2 µg/µL of Butyrophenone, otherwise 0.1 µg/µL of each) 1. Acetanilide 2. Acetophenone 3. Propiophenone 4. Butyrophenone 5. Benzophenone 6. Valerophenone 7. Hexanophenone 8. Heptanophenone 9. Octanophenone		
Solvent:	A = Water, B = ACN (not filtered)		
Temperature:	40 °C	95 °C	40 °C
Column:	2.1 mm x 50 mm, 1.8 µm	2.1 mm x 50 mm, 1.8 m	4.6 mm x 150 mm, 5.0 µm
Flow:	1.0 mL/min	2.4 mL/min	1.2 mL/min
Gradient:	0.00 min 35 %B 0.90 min 95 %B 1.10 min 95 %B 1.11 min 35 %B	0.00 min 35 %B 0.38 min 95 %B 0.45 min 95 %B 0.46 min 35 %B	0.00 min 35 %B 10.80 min 95 %B 13.20 min 95 %B 13.21 min 35 %B
Stop time:	1.15 min	0.48 min	13.20 min
Post time:	0.70 min	0.29 min	9.20 min
Wavelength:	Signal 245 nm (8) Reference 450nm (100)	Signal 245nm (8) Reference 450nm (80)	
Slit:	8 nm	8 nm	
Peak width:	> 0.0025 min (0.05 s response time) 80 Hz	> 0.01 min (> 0.2 s) 20 Hz	
Spectra:	All, 190-500 nm BW=1 nm	All 190-900 nm BW=2 nm	
Injection volume:	1 µL	1 µL	5 µL (not scaled)
Injector:	Overlapped injection Automatic delay volume reduction, Sample flush out factor = 10 Needle wash = 5 s	same	same

Resolution

For some application areas temperature becomes a further parameter in the optimization of the separation of co-eluting peaks. Figure 2 shows separations run at 40 °C and 72 °C column temperature. At 40 °C peak 3 and 4 co-elute whereas at 72 °C the peaks are separated. From the chromatograms in figure 2 it can be clearly seen that increasing the column temperature also reduces the analysis time from about 6 min to 3.5 min. In this application example there were gains in speed and resolution. Elevated column temperatures can also be used for complex samples. In some cases the resolution and the number of separated peaks can be increased using higher column temperatures (figure 3). When analyzing the Ginseng extract at temperatures as high as 80°C and 100 °C the peak clusters showed better resolution than at 60 °C.

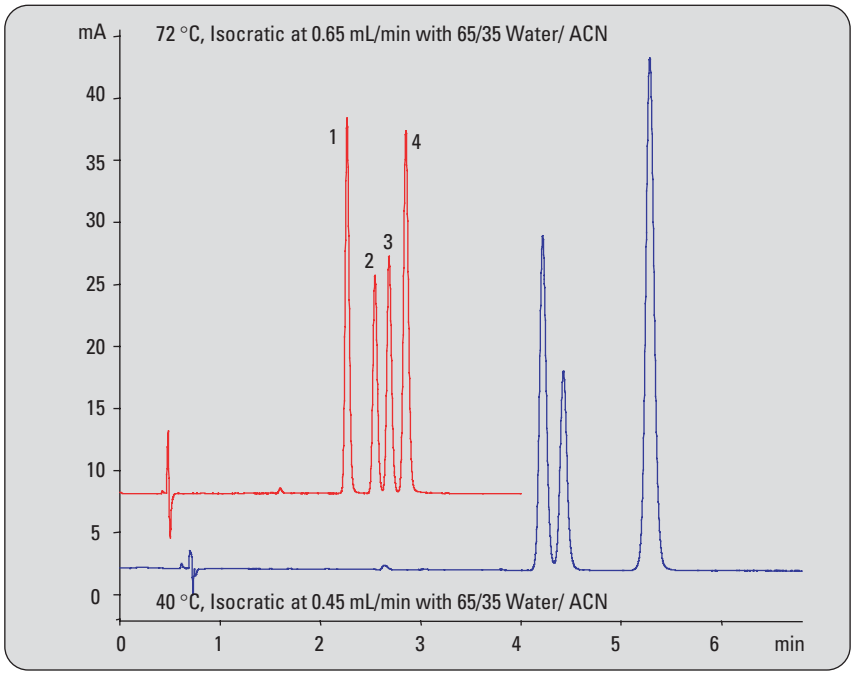


Figure 2
Influence of column temperature on separation.

Chromatographic conditions

Test sample:	Set of 4 compounds, dissolved in ACN 1. Chlortoluron 40 ng/µL 2. Atrazine 60.6 ng/µL 3. Diuron 30 ng/µL 4. Isoproturon 80 ng/µL
Column:	150 x 2.1 mm ZORBAX SB C-18, 1.8 µm for 600 baroperation
Solvent:	Water/ACN = 65/35
Flow rate:	0.65 mL/min and 0.45 mL/min
Injection volume:	1 µL, wash exterior of needle for 5 s
Temperature:	varied
Detection:	Signal: 245/10 nm, reference 450/100 nm 2 µL cell 20-Hz data rate Peak width = >0.01 min Slit width: 4 nm
Configuration:	Agilent 1200 Series Rapid Resolution LC system, standard set up, with column connected directly to the detector cell

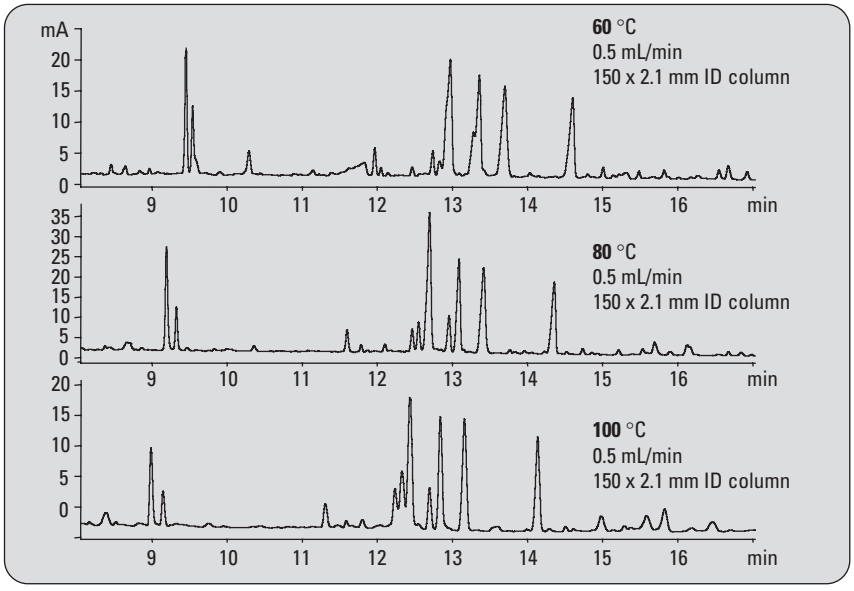


Figure 3
Ginseng extract analyzed at different column temperatures.

Chromatographic conditions

Sample:	Ginseng extract
Column:	150 x 2.1 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation
Solvent:	A = Water, B = ACN
Gradient:	10 to 95 %B in 30 min
Stop time:	30 min
Post time:	8 min
Flow rate:	0.5 mL/min
Injection volume:	5 µL, wash exterior of needle for 10 s
Temperature:	60 °C, 80 °C and 100 °C
Detection:	Signal: 210/8 nm, 230 nm, 280/10 nm, ref. 360/80 nm 2 µL cell 20 Hz data acquisition Peak width = >0.01 min Slit width: 8 nm

Chromatographic conditions

Sample:	Test mixture containing Uracil, Caffeine, Pyridin, Phenol, Aniline, Acetophenone, Propylparabene, Benzene and Toluene
Column:	150 x 4.6 mm ZORBAX SB C-18, 1.8 µm, 600 bar
Solvent:	Water/acetonitrile = 60/40 and 50/50 isocratic
Injection volume:	2 µL
Detection:	Signal 210/8 nm, reference 360/100 nm Slit width 4 nm 20 Hz data acquision

Peak shape

Basic compounds such as pyridine often show tailing effects, which are often avoided by adding appropriate modifiers to the mobile phase. Another possibility is to increase the column temperature as shown in figure 4. Using 80 °C instead of 40 °C as temperature for the column compartment significantly reduced the tailing of the pyridine peak. The following two peaks are now baseline separated, which led to better and more reliable quantitative results.

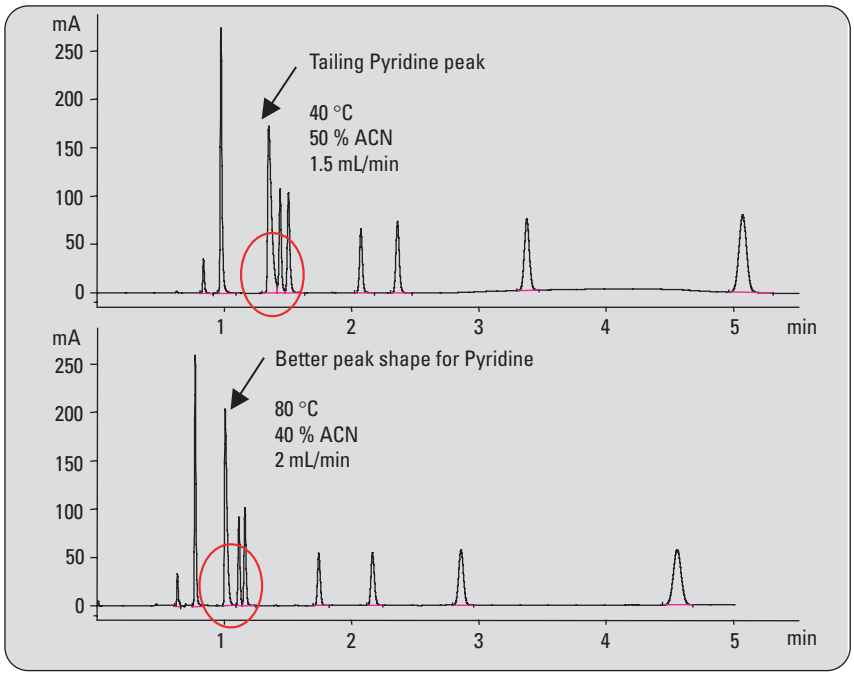


Figure 4
Influence of temperature on peak shape.

Conclusions

Increasing the column temperature can definitely help to improve speed of analysis, separation and peak shape.

- The **speed of analysis** benefited from the decrease in solvent viscosity when the column temperature was increased. The backpressure decreased and consequently the flow rate could be increased and speed of analysis was improved significantly.
- The **separation** was also influenced positively, for example, for the separation of pesticides. Here the effect of temperature on mass transfer was again responsible for the different behavior of the molecules. Also for complex samples the increase of temperature led to a better separation of peak clusters as shown for the analysis of a Ginseng extract at different column temperatures.
- The **peak shape** was also influenced positively as shown here for the analysis of pyridine, which has a strong basic character. Typically a buffer system would be used to improve the peak shape of this basic compound.

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