

# **Fast and Ultra-fast Analysis with the Agilent 1200 Series Rapid Resolution LC System Compared to a Conventional Agilent 1100 Series LC System Using Sub 2- $\mu$ m Particle Columns**

## **Application Note**

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### **Abstract**

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges and lower delay volume - predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns. This Application Note shows that shorter run times, shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained using the Agilent 1200 Series Rapid Resolution LC (RRLC) system.



**Agilent Technologies**

## **Introduction**

Due to an increasing workload in many analytical laboratories, a need to develop analytical methods faster has arisen. Furthermore, developing faster methods for standard columns is critical. Increasingly more applications are carried out using LC/MS systems, therefore there is also a demand to use narrow-bore columns for full compatibility with most MS engines. Narrow-bore columns with an internal diameter of 2.1 mm and lower have high demands in respect to low delay volumes and dispersion volumes before and after the column. In the following experiment an example is given, showing how fast methods can be developed on an LC system taking advantage of higher pressure and temperature limits of state-of-the-art equipment. In addition, speed and performance comparisons are made between a conventional Agilent 1100 Series LC system and an Agilent 1200 Series Rapid Resolution LC system, using 4.6-mm ID columns and 2.-mm ID columns packed with 1.8- $\mu$ m particles.

## **Experimental**

An Agilent 1200 Series RRLC system was used with the following modules:

- Agilent 1200 Series binary pump SL with vacuum degasser for applications using 1.8- $\mu$ m particle columns up to 150-mm length and with internal diameters from 2.1 to 4.6 mm
- Agilent 1200 Series high-performance autosampler SL for highest area precision
- Agilent 1200 Series thermostatted column compartment SL with wide temperature range from 10 degrees below ambient 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 50-mm length, packed with 1.8- $\mu$ m particles
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number G1316-68744)

An Agilent 1100 Series LC system was used with the following modules:

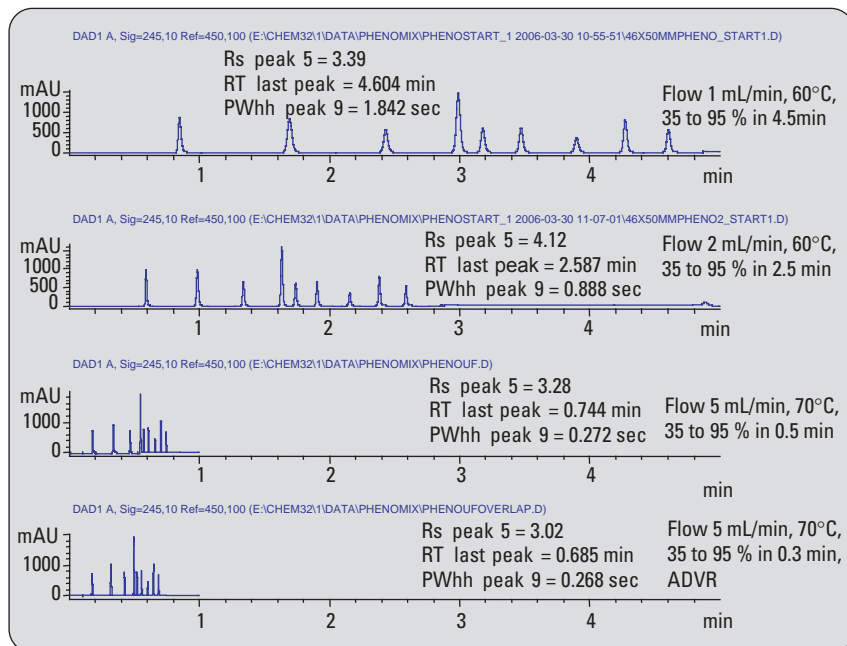
- Agilent 1100 Series binary pump with vacuum degasser
- Agilent 1100 Series well-plate autosampler
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode-array detector B
- Low dispersion kit for optimized conditions for 2.1-mm ID columns (Agilent part number 5065-9947)

## **Results and discussion**

In the past the Agilent 1100 Series LC system was frequently used for fast and ultra-fast analysis<sup>1</sup>. The instrument is very well suited specifically for the analysis of compounds using short 4.6-mm ID column packed with 1.8- $\mu$ m particles, and run times below one minute. Cycle times below two minutes were achieved. The Agilent 1200 Series RRLC system is a newly developed LC system with a wider pressure and temperature range, lower system delay volumes and improved noise for the DAD system. Due to these advancements, speed and performance have improved compared to an Agilent 1100 Series LC system, especially for columns with an internal diameter of 2.1 mm.

### Experiments using a 4.6-mm ID column

Both instruments were set up in a standard configuration with mixers and 0.17-mm ID flow capillaries installed. Typically the same parameters can be used to optimize an LC method for speed and resolution. These parameters are flow rate, column temperature, gradient profile and other instrument-specific parameters such as switching the autosampler delay volume out of the flow path after the sample has reached the top of the column (ADVR=automatic delay volume reduction). Gradient changes can therefore reach the column much faster. A typical example of how a fast method can be developed is given in figure 1. The objective is to achieve fast cycle times and a minimum resolution of 2 for all peaks.



**Figure 1**  
**Method development of an ultra fast LC method.**

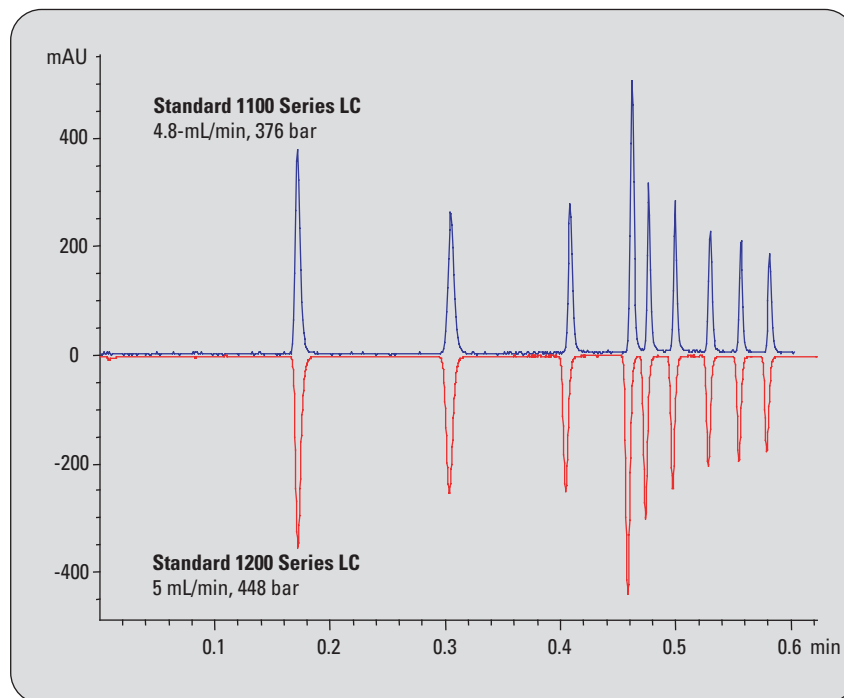
#### Chromatographic conditions:

Test sample:	Set of 9 compounds; 100 ng/μL each; dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3: Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6.Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	50 x 4.6 mm ZORBAX SB C-18, 1.8 μm for 600 bar operation
Pump:	Solvent A: H <sub>2</sub> O + Solvent B: ACN Gradient: 35 to 95 % B using different profiles
Autosampler:	Injection volume: 1 μL Wash 5 sec for needle exterior flush out factor 20
Thermostatted column compartment:	Temperature: different temperatures
Diode array detector	B and diode-array detector SL:
	Signal: 245/10 nm Ref 450/100 nm

Optimization of all of the above-mentioned parameters on both systems resulted in the chromatograms shown in figure 2. The pressure limit of 400 bar on the Agilent 1100 Series LC system restricts the maximum possible flow. 5 mL/min flow was not possible, even though the column temperature was set to 80 °C, which is the upper limit for the 1100 Series column compartment. The Agilent 1200 Series RRLC system can be operated with up to 600 bar and up to 100 °C. Applying a flow rate of 5 mL/min can be done without reaching the 600 bar pressure limit at elevated temperatures. In addition, due to design changes, the noise level of the Agilent 1200 Series DAD SL has significantly improved compared to the Agilent 1100 Series DAD B.

The performance for both systems is shown in table 1.

Resolution and noise have improved with the Agilent 1200 Series RRLC system, whereas run and cycle times are comparable. The noise level of the 1200 Series RRLC system can be further reduced using the post column cooling device<sup>2</sup>. The device adapts the temperature of the column effluent to the temperature of the optical unit. This further reduces the noise level, especially if high flow rates and high temperatures are used. Another possibility to reduce cycle time is to enable the overlapped injection features, which is possible with both systems.



**Figure 2**  
Standard Agilent 1200 Series RRLC system vs. Agilent 1100 Series LC system: analysis of phenone mix on 4.6-mm ID column packed with 1.8- $\mu$ m particles.

#### Chromatographic conditions:

Test sample:	Set of 9 compounds, 100 ng/ $\mu$ L each, dissolved in water/ACN (65/35) 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone
Column:	50 x 4.6 mm ZORBAX SB C-18, 1.8 $\mu$ m for 600 bar operation
Pump:	Solvent A: H <sub>2</sub> O, Solvent B: ACN Gradient: 35 to 95 % B in 0.3 min
Autosampler:	Injection volume: 1 $\mu$ L Wash 5 sec for needle exterior, flush-out factor 20
Thermostatted column Compartment:	Temperature: 80 °C
Detector DAD B and DAD SL:	Signal: 245/10 nm Ref 450/100 nm

Parameter	Standard 1100 Series 80 °C 4.8 mL/min	Standard 1200 Series 80 °C 5 mL/min
Flow rate	4.8 mL/min	5 mL/min
Run time	0.60 min	0.60 min
Cycle time	1 min 37 sec	1 min 37 sec
Rs Peak 5	2.22	2.30
PW1/2 peak 9	0.00378 min	0.00375 min
PW1/2 peak 1	0.00458 min	0.00486 min
Noise PtoP	6.2021 mAU	0.7930 mAU
Backpressure	376 bar	448 bar
Injection volume	1 $\mu$ L	1 $\mu$ L
DAD data rate	20 Hz, path 10 mm	80 Hz, path 10 mm

**Table 1**  
Performance comparison for 4.6-mm ID column.

Furthermore, column switching valves can be installed in the ovens, which provides even higher sample throughput using 2 columns for analysis. A sample is analyzed on the first column, while the second column is regenerated using a second pump. If the analysis on the first column is completed, the next injection can be immediately performed on the previously equilibrated second column.

### Experiments using 2.1-mm ID column

Columns with an internal diameter of 2.1 mm and lower have high demands regarding low delay volumes and dispersion volumes before and after the column. Using columns with an internal diameter of 2.1 mm, the Agilent 1100 Series binary LC system must be optimized without using a mixer or only a mixer with a significantly smaller volume and capillaries with smaller IDs for all flow connections. Nevertheless, cycle times below 2 minutes could barely be achieved using columns packed with 1.8  $\mu\text{m}$  particles and 50 mm length. This was mainly due to the pressure limitation of 400 bar for the Agilent 1100 Series LC system. In addition, the delay volume of the 1100 Series LC system is a drawback for fast run and equilibration times. With the introduction of the Agilent 1200 Series RRLC system this gap was closed. Now using narrow bore columns packed with 1.8- $\mu\text{m}$  particles, run times below 0.5 min are possible, with higher flow rates and

elevated temperatures. Both systems are compared using the same column and optimized instrument configurations. To allow for optimized conditions for both systems, the following set-ups were used:

#### Configuration of the Agilent 1100 Series LC system:

- The mixer was replaced by a short capillary with an internal diameter of 0.12 mm (Agilent part number G1312-67301)
- Seat and seat capillary were replaced by 0.12-mm ID parts (well-plate seat, Agilent part number G1367-87104, and seat capillary, Agilent part number G1313-87103)
- The capillary from the injector to the column compartment was replaced with a 0.12-mm ID capillary (Agilent part number 01090-87610)
- The 0.17-mm ID capillary from the column compartment to the column was exchanged with a capillary with an internal diameter of 0.12 mm (Agilent part number G1316-87303)
- The column was connected to the detector using the detector inlet capillary.
- A 1.7- $\mu\text{L}$  cell with a path length of 6 mm was used as the detector cell.

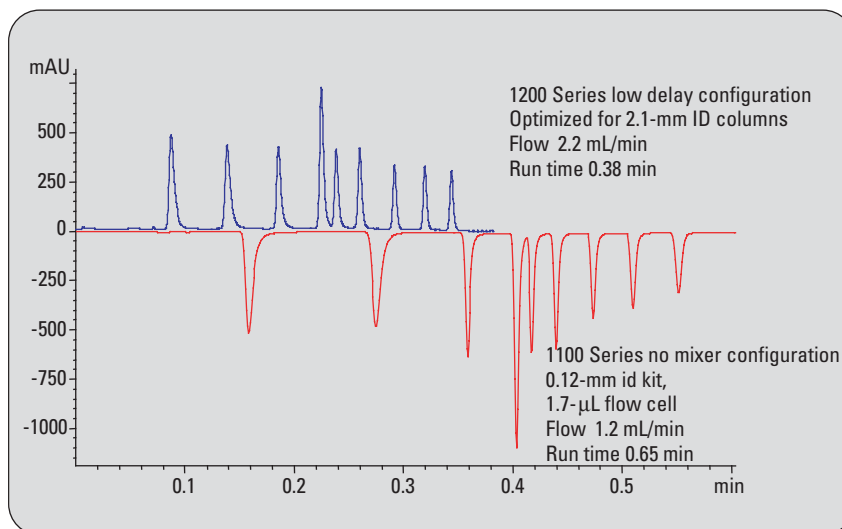
#### Configuration of the Agilent 1200 Series RRLC system:

- The low delay volume configuration for the pump was set up with a 120- $\mu\text{L}$  delay volume (mixer and damper were moved out of the flow path).
- Two flow capillaries were replaced with 0.12-mm ID capillaries, all included in the Agilent 1200 Series low dispersion kit (Agilent part number G1316-68744).
- The seat capillary was also replaced with a 0.12-mm ID capillary (included in kit Agilent part number G1316-68744)
- The DAD SL 2  $\mu\text{L}$  flow cell with a 3-mm path length was used. The inlet capillary was directly connected to the column outlet.

The same 2.1 x 50 mm column was used for both systems. The flow rate was set so that the back-pressure was close to the limit of each system. Automated delay volume reduction (ADVR) was selected in the injector setup screen for both systems. The injection volume was set to 1  $\mu\text{L}$  for the Agilent 1100 Series LC system, and to 2  $\mu\text{L}$  for the Agilent 1200 Series RRLC system to compensate for the lower path length of the 1200 Series 2- $\mu\text{L}$  flow cell.

In figure 3 an overlay of the chromatograms obtained from both systems is shown. In table 2 the performance for both system is recorded.

The chromatograms in figure 3 clearly demonstrate the advantages of the Agilent 1200 Series RRLC system, using 2.1-mm ID columns, packed with 1.8- $\mu$ m particles. Faster run times and cycle times are possible, due to the fact that higher flow rates can be obtained with the Agilent 1200 Series RRLC system. Table 2 indicates that the cycle time for the Agilent 1200 Series RRLC system is only half that of the Agilent 1100 Series LC system. In addition, the resolution of the 5th peak and also peak width at half height is significantly improved at higher flow rates.



**Figure 3**  
Analysis performed with a 2.1-mm ID column with the optimized Agilent 1200 Series RRLC system and the optimized Agilent 1100 Series LC system using automated delay volume reduction for both systems.

#### Chromatographic conditions:

Test sample: Set of 9 compounds, 100 ng/ $\mu$ L each, dissolved in water/ACN (65/35)  
1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone,  
5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone,  
9. Octanophenone

Column: 50 x 2.1 mm ZORBAX SB C-18, 1.8  $\mu$ m for 600 bar operation

Pump: Solvent A: H<sub>2</sub>O, Solvent B: ACN  
Gradient: 35 to 95 % B in 0.3 min

Autosampler: Injection volume: 1 and 2  $\mu$ L  
Wash 5 sec for needle exterior, flush out factor 20

Thermostatted column compartment:  
Temperature: 80 and 95  $^{\circ}$ C

Detector DAD B and DAD SL:  
Signal: 245/10 nm Ref 450/100 nm

Parameter	1100 Series, optimized, no mixer, ADVR, 80 $^{\circ}$ C	1200 Series, optimized, low delay volume configuration, ADVR, 95 $^{\circ}$ C
Flow rate	1.2 mL/min	2.2 mL/min
Run time	0.65 min	0.38 min
Cycle time	2 min 33 sec	1 min 16 sec
Rs Peak 5	1.86	2.15
PW1/2 peak 9	0.00556 min	0.00328 min
PW1/2 peak 1	0.00729 min	0.0049 min
Noise PtoP	0.1 mAU	0.2 mAU
Backpressure	370 bar	570 bar
Injection volume	1 $\mu$ L	2 $\mu$ L
DAD data rate	20 Hz, path 6 mm	80 Hz, path 3 mm

**Table 2**  
Performance comparison using a 2.-mm ID column.

## **Conclusions**

Faster method development for faster LC methods is a requirement that can be met with state-of-the-art LC equipment. Even though conventional LC equipment can also provide fast methods, better performance and time savings can be obtained on specially designed LC systems with wider pressure and temperature ranges. Predominantly with 2.1-mm ID columns, where typically lower flow rates are used than on 4.6-mm ID columns, an LC system like the Agilent 1200 Series RRLC system provides significantly lower delay volumes. Shorter run times and shorter equilibration times, and consequently shorter cycle times and more sample throughput are obtained.

## **References**

1. Anabel Fandino, "Ultra-fast liquid chromatography using the Agilent 1100 Series HPLC system and 1.8-um ZORBAX SB C18 Rapid Resolution HT columns", *Agilent Application Note, publication number 5989-1603EN*, **2004**.
2. A.G.Hüsken, "Agilent 1200 Series column compartment SL with temperature control up to 100 °C and post-column cooling for lowest baseline noise", *Agilent Application Note, publication number 5989-5034EN*, **2006**.

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