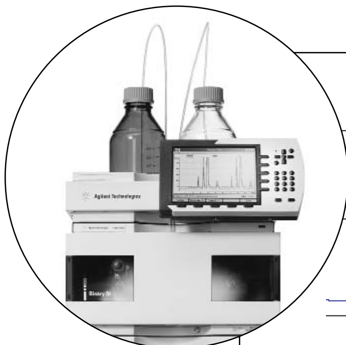
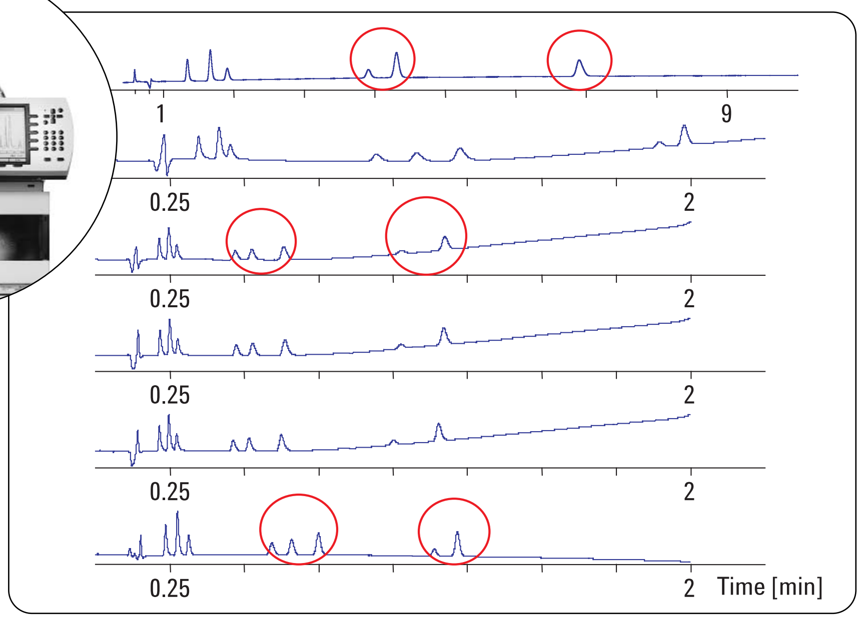
Step-by-step upgrade of Agilent 1100 Series LC systems to Agilent 1200 Series Rapid Resolution LC systems for higher performance

Part 1: Optimizing for use with 2.1 mm ID columns

Technical Note





Abstract

Obtaining faster results from a sample, and discovering what you could not detect before, is the result of Agilent's Rapid Resolution Liquid Chromatography technology. As the owner of an Agilent 1100 Series LC system you can step up to this performance with an investment tuned to your budget and your requirements. The level of compatibility between the Agilent 1100 Series LC and the Agilent 1200 Series LC systems facilitates a step-by-step upgrade from an Agilent 1100 Series system to an Agilent 1200 Series RRLC system that is fully configured to provide:

- Shorter run and cycle times for increased productivity
- More resolution for more certainty of not missing impurities
- Improved signal-to-noise ratios for lower detection limits

Agilent Equipment:

1100 LC Series LC
1200 Series SL modules
1200 Series Rapid Resolution LC system

Agilent Technologies

Introduction

In 1995, the Agilent 1100 Series LC system was introduced and up to 2005 more than 60,000 systems and 300,000 modules were sold worldwide. The Agilent 1100 Series LC system established itself as the world's leading LC system and was known for its excellent performance and robustness. In 2006, the Agilent 1200 Rapid Resolution LC series was introduced. This new system from Agilent was specifically designed to support short and long columns packed with sub-two micron particles. The combination of optimized LC equipment and columns packed with sub-two micron particles can achieve the same or even better performance in shorter times.

This Technical Note describes how an Agilent 1100 Series LC system can be upgraded step-by-step to a fully equipped Agilent 1200 RR LC system. For each individual step the gain in performance is shown. The step-by-step procedure was performed using 2.1 mm ID columns, whereby the 1100 and 1200 Series systems used were optimized for this type of column.

Instrumentation

Initial configuration of Agilent 1100 Series LC system for step 1:

- Agilent 1100 Series binary pump, well-plate sampler, thermostatted column compartment, diode array detector with 1.7 µl flow cell, 0.12 mm ID flow capillaries and seat capillary, no mixer

Configuration for step 2:

- Detector replaced by Agilent 1200 Series diode array detector SL

Configuration for step 3:

- Column compartment replaced by Agilent 1200 Series thermostatted column compartment SL

Final configuration of Agilent 1200 Series RRLC system for step 4:

- Agilent 1200 Series binary pump SL in low-delay configuration (mixer and damper out of flow path for lowest delay volume)
- Agilent 1200 Series thermostatted column compartment SL with small heat exchanger (1.6 µl internal volume)
- Agilent 1200 Series diode array detector SL with 2 µl flow cell (detection wavelength 204/10 nm, reference wavelength 350/80 nm, slit width 8 nm)
- Low dispersion kit – all flow capillaries and seat capillary with 0.12 mm ID

Results and discussion

Several steps were performed to evaluate the gain in performance by replacing columns and modules:

Step 1: Replacement of 150 mm, 2.1 mm ID, ZORBAX SB C18 column packed with 5 µm particles with 50 mm, 2.1 mm ID, ZORBAX SB C18 column packed with 1.8 µm particles on Agilent 1100 Series LC system optimized for 2.1 mm ID columns

Step 2: Replacement of detector with Agilent 1200 Series diode array detector SL

Step 3: Replacement of column compartment with Agilent 1200 Series thermostatted column compartment SL

Step 4: Fully equipped Agilent 1200 Series Rapid Resolution LC system optimized for 2.1 mm ID columns

Sample	1. Phenylethylmalonamide 2. Ethosuximide 3. Primidone 4. Phenobarbital 5. Methylphenylsuccimide 6. Carbamazepine-epoxide 7. Phenytoin 8. Carbamazepine
Stock solution	1:100 dilution, 3.9 to 39.3 ng / 3 µL
Mobile phase	Water with 100 µL/L TFA and Acetonitrile with 75 µL/L TFA
Columns	2.1 x 150 mm ZORBAX SB C18, 5 µm in initial configuration 2.1 x 50 mm ZORBAX SB C18, 1.8 µm in steps 1 through 4

Step 1:
Replacing the column

In the first step, the 150 mm, 2.1 mm ID, ZORBAX SB C18 column packed with 5 µm particles was replaced by a 50 mm, 2.1 mm ID, ZORBAX SB C18 column packed with 1.8 µm particles. The Agilent 1100 Series LC system was optimized for use with 2.1 mm ID columns. The introduction of columns packed with 1.8 µm particles by Agilent in 2002 was a big step forward to achieve the same or even better performance in shorter time. The theory behind this is well known and was proven in several publications[1,2,3,4]. This issue will not be discussed in this note and the results shown in figure 1 again confirm the benefits of using columns packed with 1.8 µm particles. The upper chromatogram shows the analysis of eight compounds using conventional conditions and a 150 x 2.1 mm ZORBAX SB C18, 5 µm column. Under the applied conditions, peaks 5 and 6 and peaks 7 and 8 coelute. The lower chromatogram shows the same analysis using a 50 x 2.1 mm ZORBAX SB C18 column packed with 1.8 µm particles. All peaks were separated and the run time was significantly lower.

Performance improvements:
- Reduction in run time from 8 to 1.4 min, an increase in speed of 83 %
- Separation of peaks 5 and 6 with a resolution of 3.30 for peak 6
- Separation of peaks 7 and 8 with a resolution of 3.99 for peak 8

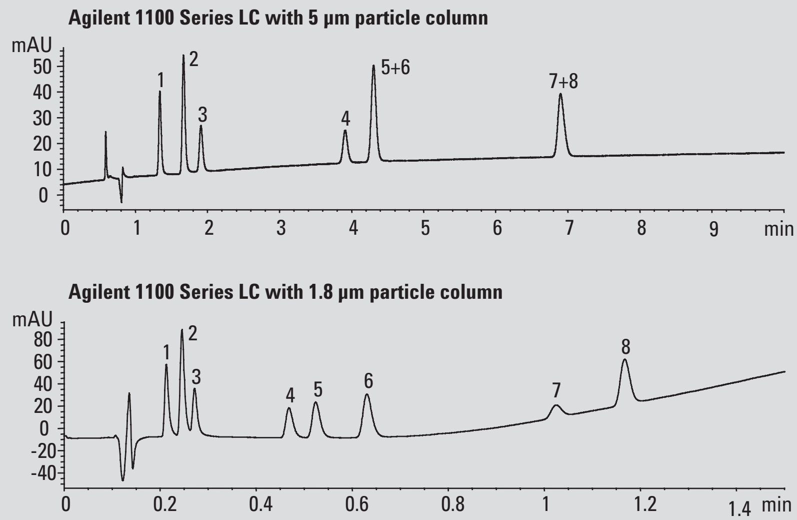


Figure 1
Step 1: Comparison of chromatograms obtained on a long column packed with 5 µm particles (upper trace) and a short column packed with 1.8 µm particles (lower trace). Both analyses were run on an Agilent 1100 Series LC system optimized for 2.1 mm ID columns.

Conditions upper trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary
Column:	150 x 2.1 mm, 5 µm
Flow rate:	0.5 mL/min
Gradient:	20 to 40 %B in 10 min
Column temperature:	40 °C
Detection:	204/10 nm, 20 Hz, 1.7 µL flow cell
Conditions lower trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	80 °C
Detection:	204/10 nm, 20 Hz, 1.7 µL flow cell

Step 2:
Replacing the detector

In a second step, the detector was replaced by the Agilent 1200 Series diode array detector SL, which acquires data at higher rates and gives better baseline performance. Higher data rates such as 40 Hz increase resolution. Figure 2 shows a comparison between the two detectors used in this study. The upper trace shows the analysis at a data rate 20 Hz and the lower trace shows the same analysis at 40 Hz. The increase in resolution was in the low percentage range. However, the signal-to-noise ratio improved significantly. The improvement in the signal-to-noise ratio was primarily a result of the new cell design in the Agilent 1200 Series diode array detector SL. The peak-to-peak noise of the Agilent 1100 Series DAD was about 0.4835 mAU. For this application the peak-to-peak noise of the Agilent 1200 Series DAD SL was 0.2928 mAU. Using the Agilent 1200 Series DAD SL also improved the resolution, resulting from the higher rate at which this detector can acquire data.

Resolution increased:
- 4 % for peak 3
- 9 % for peak 5
- 13 % for peak 8

Signal-to-noise ratio improved:
- 52 % for peak 3
- 56 % for peak 5
- 67 % for peak 8

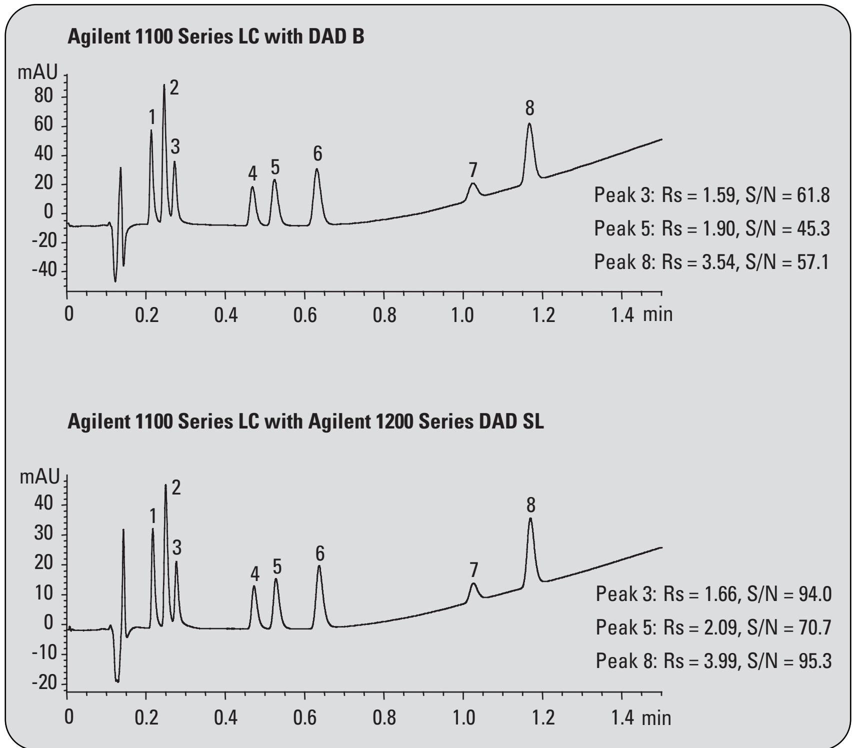


Figure 2
Step 2: Comparison of Agilent 1100 Series DAD B with 20 Hz data rate and Agilent 1200 Series DAD SL with 40 Hz under the same chromatographic conditions.

Conditions upper trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	80 °C
Detection (DAD B):	204/10 nm 20 Hz, 1.7 µL flow cell
Conditions lower trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary, Agilent 1200 Series DAD SL
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	80°C
Detection (DAD SL):	204/10 nm, 40 Hz, 2 µL flow cell

Step 3:
Replacing the column compartment

In step 3, the Agilent 1200 Series thermostatted column compartment SL was integrated in the Agilent 1100 Series LC system. This new column compartment is designed to operate up to 100 °C and to maintain more stable temperatures. To reduce extra column volume further a new heat exchanger was installed. This heat exchanger had an internal volume of 1.6 µL. In terms of resolution, using the new column compartment improved performance significantly. This improvement in resolution resulted from the lower delay volume of the heat exchanger, which reduced peak dispersion.

Signal-to-noise ratios were about the same.

Increases in resolution:

- 47 % for peak 3
- 11 % for peak 5
- 21 % for peak 8

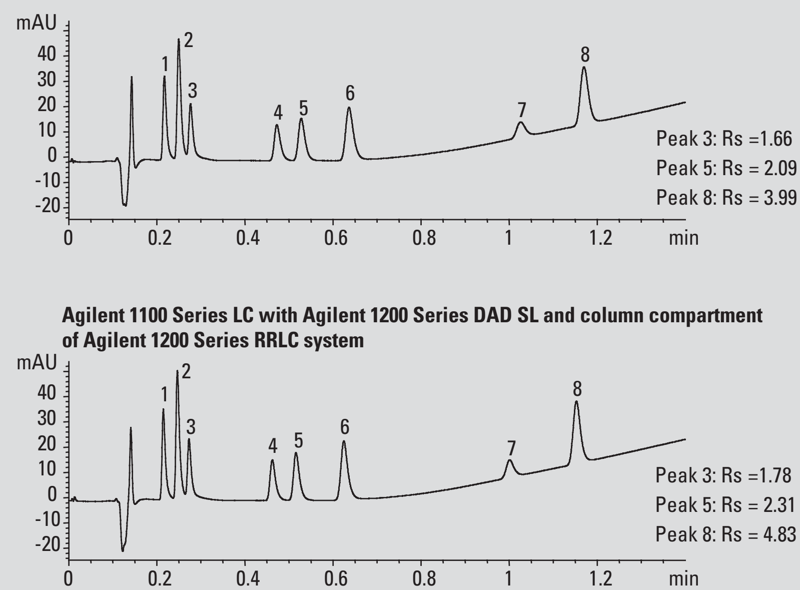


Figure 3
Step 3: Comparison of performance between Agilent 1100 Series and 1200 Series thermostatted column compartments.

Conditions upper trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary, Agilent 1200 Series DAD SL
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	80 °C
Detection (DAD SL):	204/10nm, 40 Hz, 2 µL flow cell
Conditions lower trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary, Agilent 1200 Series DAD SL, Agilent 1200 Series thermostatted column compartment with small heat exchanger
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 B% in 1.5 min
Column temperature:	80 °C
Detection (DAD SL):	204/10 nm, 40 Hz, 2 µL flow cell

Step 4:
Moving to a fully equipped Agilent 1200 Series RRLC system

The upgrade to a fully equipped Agilent 1200 Series RRLC system was completed by integration of a 1200 Series binary pump SL and a 1200 Series high performance autosampler SL. The system was now able to take full advantage of the RRLC design and performance. Integration of the binary pump or autosampler on their own did not make sense because both modules are required to take full advantage of the enhanced pressure range of up to 600 bar. The delay volume of the system is adaptable to the needs of columns with small internal diameters. If the mixer and damper are taken out of the flow path, the delay volume is about 120 µL. This ensures that gradient changes reach the top of the column quickly. A further improvement is the availability of a low dispersion kit that allows the user to replace all flow capillaries with 0.12 mm ID capillaries. The seat capillary can also be replaced with a 0.12 mm ID capillary. The post-column volume can be reduced by using the new 2 µl flow cell for the DAD SL. This cell has a path length of 3 mm. All these enhancements can significantly reduce the extra column volume. Using the Agilent 1100 Series LC system with 1200 Series DAD SL and thermostatted column compartment SL, a flow rate of 1 mL/min was reached at a column temperature of 80 °C. The backpressure was 328 bar. Using the Agilent 1200 Series RRLC system, the column temperature was lowered to 60 °C and the 1 mL/min flow rate was still maintained at a backpressure of 430 bar. The results show that some peaks were temperature dependent. Lowering the temperature was advantageous for peaks 3 and 5 in terms of resolution. In contrast, a higher temperature was better for peaks 7 and 8. Nevertheless, the resolution of Rs = 2.87 for both peaks was still sufficient.

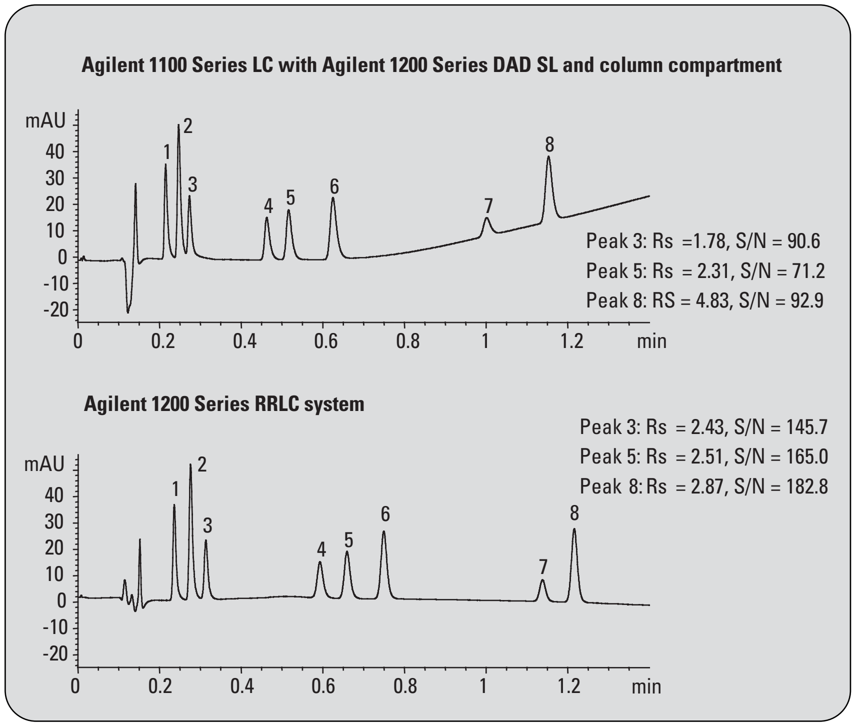


Figure 4
Step 4: Comparison between an Agilent 1100 Series LC with Agilent 1200 Series DAD SL and column compartment, and an Agilent 1200 Series RRLC system optimized for 2.1 mm ID columns.

Conditions upper trace:	Agilent 1100 Series LC system, no mixer, 0.12 mm ID flow capillaries and seat capillary, Agilent 1200 Series DAD SL, Agilent 1200 Series thermostatted column compartment with small heat exchanger
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	80 °C
Detector (DAD SL):	204/10 nm, 40 Hz, 2µL flow cell
Conditions lower trace:	Agilent 1200 Series RRLC system, low delay volume configuration, 0,12 mm ID flow capillaries and seat capillary
Column:	50 x 2.1 mm, 1.8 µm
Flow rate:	1 mL/min
Gradient:	20 to 40 %B in 1.5 min
Column temperature:	60°C
Detection (DAD SL):	204/10 nm, 40 Hz, 2µL flow cell

Performance improvements in terms of resolution:
- 37 % for peak 3
- 9 % for peak 5

Signal-to-noise improved:
- 60 % for peak 3
- 61 % for peak 5
- 97 % for peak 8

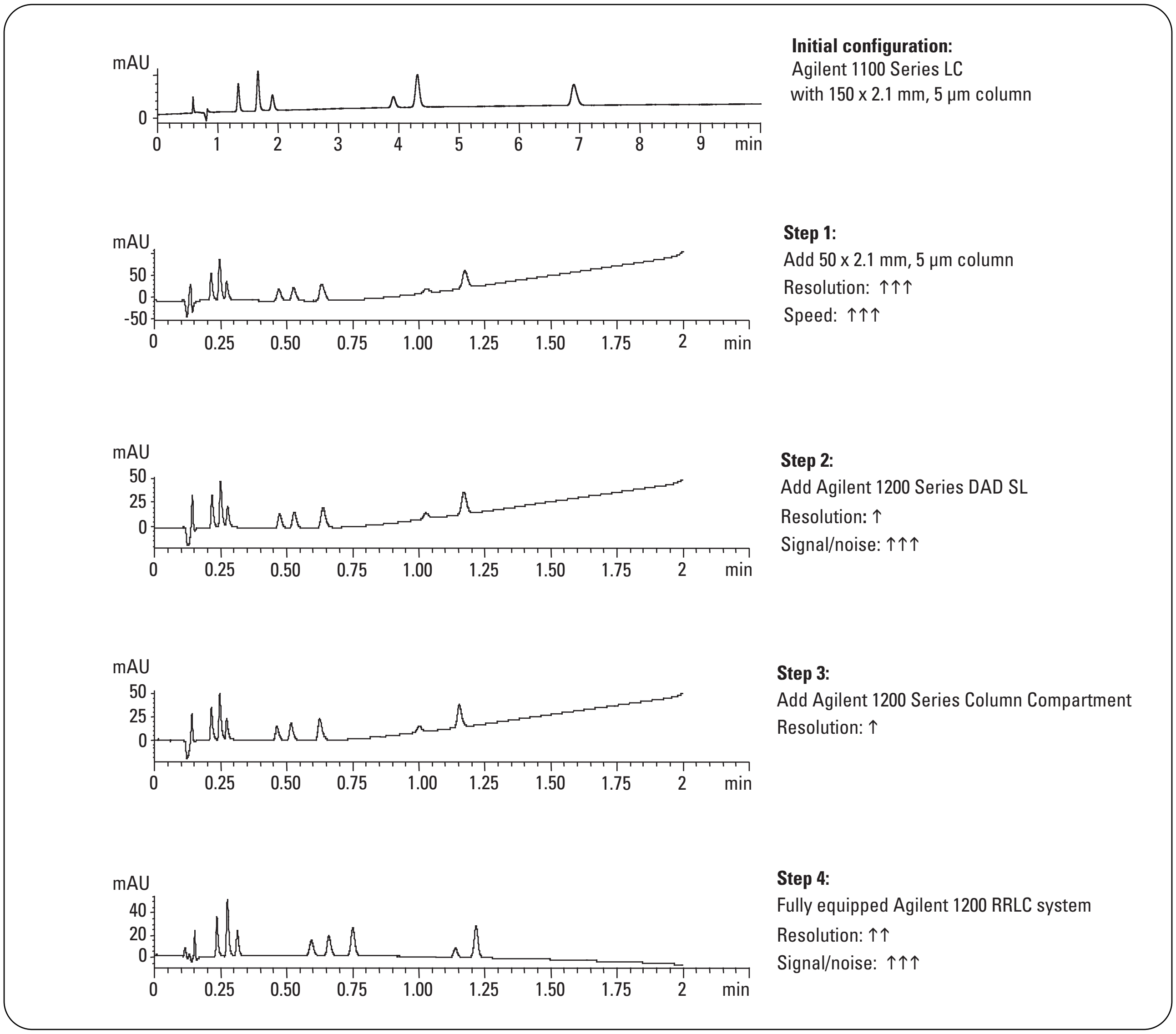


Figure 5
Improving performances by upgrading the Agilent 1100 Series LC step-by-step to the Agilent 1200 Series RRLC system.

Conclusion

The Agilent 1100 Series LC system can be upgraded step-by-step to a fully equipped Agilent 1200 Rapid Resolution LC system, providing more speed, resolution and a better signal-to-noise ratio, (figure 5). The stepwise upgrade to the Agilent 1200 Series RRLC system gives benefits at each individual step:

- Step 1: Using short columns packed with 1.8 µm particles gives a significant increase in speed and resolution.
- Step 2: Adding the Agilent 1200 Series DAD SL increases the signal-to-noise ratio significantly and also improves resolution.
- Step 3: Adding the 1200 Series thermostatted column compartment improves resolution further.
- Step 4: Moving to a fully equipped Agilent 1200 Series RRLC system increases the signal-to-noise ratio significantly and for most peaks the resolution is also enhanced.

References

1.
A. D. Jerkovich, J. Scott Mellors, James W. Jorgenson, “The Use of Micron-Sized Particles in Ultrahigh-Pressure Liquid Chromatography”, *Recent Developments in LC Column Technology*, *www.lcgceurope.com*, *June* **2003.**

2.
Ronald E. Majors, “Are you Getting the Most out of your HPLC Column”, *LC/GC North America*, *Volume 21 number 12*, **2003.**

3.
A.D. Broske, R.D. Ricker, B.J. Permar, Wu Chen, M.J. Joseph, “The Influence of Sub-two Micron Particles on HPLC Performance”, *Agilent Application Note*, *Publication Number 5988-9251EN*, **2003.**

4.
W.E. Barber, M.J. Joseph, “High Efficiency, High Throughput LC and LC/MS Applications Using ZORBAX Rapid Resolution HT Columns”, *Agilent Application Note*, *Publication Number 5989-0540EN*, **2004.**

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