Easy Transfer of Standard HPLC Methods to the Agilent 1200 Series Rapid Resolution LC System

Technical Note

Abstract

The first questions when the necessity for new equipment arises are:

- Are methods transferable?
- How much time and effort is needed?
- Is revalidation required?

In this Technical Note the performance of an isocratic and a gradient analysis using an Agilent 1200 Series Rapid Resolution LC system is compared to a standard Agilent 1100 Series system. Several different configurations of the 1100 Series systems were compared to the 1200 Series Rapid Resolution LC system and recommendations are given on what to consider for maintaining the same or better performance.

The conclusion is, that an established, validated method can be transferred easily to the 1200 Series Rapid Resolution LC system without altering the performance. The new 1200 Series system can replace the 1100 Series system today with no or minimal revalidation effort (while concurrently preparing for tomorrow's ultra-fast methods). The configurable delay volume of the 1200 Series binary pump SL significantly facilitates the ease of transfer.

Agilent Technologies

Introduction

The Agilent 1200 Series Rapid Resolution LC (RRLC) system[1] was designed to provide highest analysis speed and resolution and at the same time keep system pressure at a minimum. Therefore, it is the ideal instrument for very fast analyses up to 20 times faster than conventional HPLC with the same or better data quality in terms of resolution, sensitivity and precision. Further, the configurable delay volume makes the 1200 Series RRLC system compatible with existing HPLC methods, which allows the easy re-use of existing, validated methods without any changes in retention times, for example. The 1200 Series RRLC system uses the same capillaries and Swaglok fittings. Hence, not only can sub-2 µm RRLC columns be used but also any columns that are used on an 1100 Series system can be connected to the RRLC system. To adjust the delay volume, a capillary simply has to be re-connected to another position. In this way, the system can easily be reconfigured to a low delay volume configuration, which makes it ready for future use with ultra-fast methods.

The first question when considering new hardware for routine pharmaceutical analysis is: How can validated methods be transferred and how much time and effort is involved? Furthermore, it is most desirable to minimize or even prevent revalidation.

In this Technical Note the performance achieved with different 1100 Series system configurations is compared to the performance of a comparable 1200 Series RRLC system using the standard delay volume configuration. For this purpose the repeatability[a] of peak area, retention time, resolution and peak width at half height was determined for an antihistaminic and an antiasthmatic drug, both of which are regarded as typical pharmaceutical samples[2,3].

Equipment

The Agilent 1200 Series LC system comprised the following modules:

- Agilent 1200 Series binary pump SL, standard delay volume configuration
- Agilent 1200 Series high performance autosampler SL
- Agilent 1200 Series thermostatted column compartment SL
- Agilent 1200 Series diode array detector SL (standard flow cell: 10-mm path length), or Agilent 1200 Series variable wavelength detector SL (standard flow cell: 10-mm path length)

This resulted in two configurations for the 1200 Series RRLC system, one using the diode array detector (DAD)[b] and one using the variable wavelength detector (VWD). Both configurations were compared to the respective 1100 Series systems.

The Agilent 1100 Series system comprised the following modules:

- Agilent 1100 Series binary pump, or Agilent 1100 Series quaternary pump
- Agilent 1100 Series well-plate autosampler (thermostatted or non-thermostatted), or Agilent 1100 Series standard autosampler (thermostatted or non-thermostatted)
- Agilent 1100 Series thermostatted column compartment
- Agilent 1100 Series diode array detector (standard flow cell: 10 mm path length), or Agilent 1100 Series variable wavelength detector (standard flow cell: 10 mm path length)

As a result four different configurations (1 – 4) of the 1100 Series were considered as non-thermostatted (A) and thermostatted (B) versions. Configuration 1 most closely matches the 1200 Series RRLC system. Each of the 1100 Series configurations 2, 3 and 4 differ from configuration 1 in only one module. For example, configuration 2 contains a standard autosampler instead of a well-plate autosampler. Standard stainless steel 0.17-mm i.d. capillaries as described in the system manuals of the individual modules were used consistently to connect the modules. The 1200 Series VWD was connected with the standard PEEK capillary as described in the 1200 Series VWD manual. The systems were controlled using Agilent ChemStation (revision B.02.01. SR1).

[a] *Repeatability* expresses the precision under the same operating conditions over a short interval of time.

[b] Technically the 1200 Series DAD resembles the 1200 Series MWD so that the results do also apply to the MWD.

Results and discussion

Four characteristics calculated by the Agilent ChemStation were used for the performance comparison – retention time (RT), peak area, peak width at half height (hereafter referred to as "peak width") and resolution. During the experiments an isocratic as well as a gradient method were run repeatedly on a 1200 Series RRLC system and on different 1100 Series system configurations. A minimum of 10 injections were done consecutively per configuration. An isocratic run (figure 1A) was performed; theobromine, theophylline, enprofylline and caffeine were analyzed on a Hypersil ODS column[2]. In the initial gradient run (figure 1B), tripelenamine, chlorpheniramine and promethacine were analyzed on a ZORBAX SB-C18 column[3]. The repeatability is obtained by averaging the results of ten consecutive runs using the Agilent ChemStation extended statistics sequence summary report.

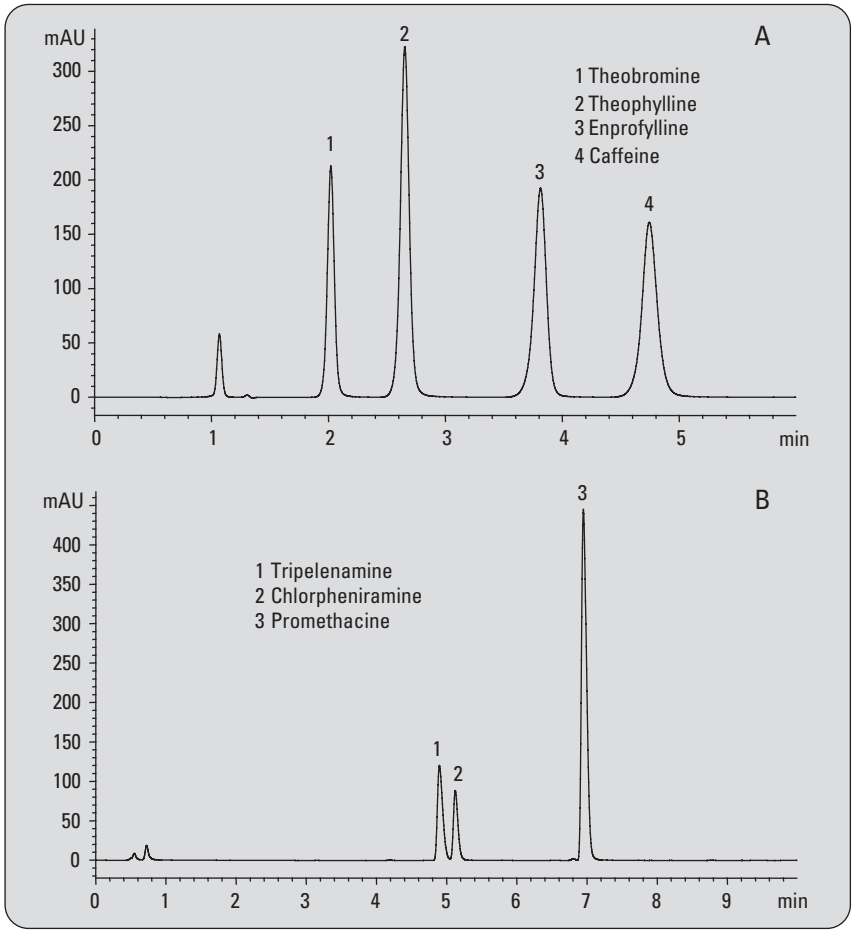

Figure 1
A) Isocratic run.
B) Gradient run.

Chromatographic conditions Figure 1A:	
Column:	Hypersil ODS 4 x 125 mm, 5 µm
Mobile phases:	Water = A
	Acetonitrile = B
Isocratic:	8 % B for 7 min
Stop time:	7 min
Flow:	1 mL/min
Injection:	5 µL
Column temperature::	50 °C
UV detector:	DAD: 270 nm/20 (ref. 360 nm/80)
	VWD: 270 nm
	Flow cell (10 mm path length)
Chromatographic conditions Figure 1B:	
Column:	ZORBAX SB-C18 4.6 x 75 mm, 5 µm
Mobile phases:	Water + 0.025 M KH_2PO_4 (pH=3) = A
	Acetonitrile = B
Gradient:	at 0 min 10 % B
	at 10 min 55 % B
	at 12 min 10 % B
Stop time:	12 min
Post time:	5 min
Flow:	1 mL/min
Injection:	5 µL
Column temperature:	25 °C
UV detector:	DAD: 255 nm/20 (ref. 380 nm/60)
	VWD: 255 nm
	Flow cell (10 mm path length)

Tables 1a to 4c list the different system configurations that were compared, followed by a discussion of the results for each configuration. For a better overview, each change compared to configuration 1 is highlighted in tables 2a, 3a and 4a..The calculated results for all compounds were the same or comparable. For better clarity, one compound was selected as an example for the isocratic and gradient mode.

Configuration 1 (table 1a) of the 1100 Series system is the closest match to the 1200 Series RRLC system. Therefore, the performance measurements produce less than a 4 % difference in relation to retention time (RT), peak area, peak width and resolution, for the gradient method (table 1b) and the isocratic method (table 1c). The variance is slightly higher for configuration 1b due to the longer capillaries required for the thermostatted well-plate autosampler. The repeatability remains equal for both gradient and isocratic methods.

CONFIGURATION 1 **1200 Series RRLC system**	**1100 Series Configuration 1A**	**1100 Series Configuration 1B**
Binary pump SL	Binary pump	Binary pump
Autosampler SL	Well-plate autosampler	Thermost. well-plate autosampler
Column compartment SL	Column compartment	Column compartment
DAD/MWD SL	DAD/MWD	DAD/MWD

Table 1a
Highly similar configuration.

GRADIENT **Chlorpheniramin**	**1200 RRLC**	**Configuration 1A**	**Deviation**	**Configuration 1B**	**Deviation**
RT [min]	5,05 ± 0,1 %	5,06 ± 0,1 %	**0,2 %**	5,08 ± 0,0 %	0,6 %
Peak width [min]	0,07 ± 0,5 %	0,07 ± 0,6 %	**1,6 %**	0,07 ± 0,5 %	1,8%
Resolution	1,76 ± 0,5 %	1,82 ± 0,5 %	**3,5 %**	1,82 ± 0,5 %	3,4 %

Table 1b
The gradient run produced these repeatability results for chlorpheniramin, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

ISOCRATIC **Theophylline**	**1200 RRLC**	**Configuration 1A**	**Deviation**	**Configuration 1B**	**Deviation**
RT [min]	2,55 ± 0,2 %	2,56 ± 0,3 %	**0,6 %**	2,59 ± 0,1 %	1,6 %
Peak width [min]	0,08 ± 0,7 %	0,07 ± 0,9 %	**0,8 %**	0,08 ± 0,6 %	0,6 %
Resolution	5,34 ± 0,5 %	5,30 ± 0,7 %	**0,8 %**	5,33 ± 0,5 %	0,2 %

Table 1c
The isocratic run produced these repeatability results for theophylline, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

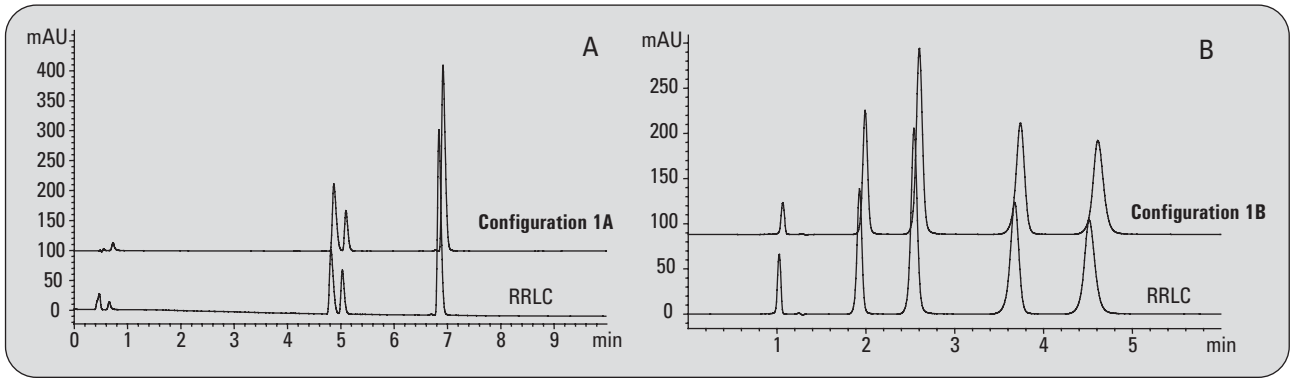

Figure 2
A) Comparison of performance, gradient run.
B) Comparison of performance, isocratic run.

In configuration 2 (table 2a) the standard autosampler was used. As the well-plate autosampler and the standard autosampler have the same internal volume, the performance (tables 2b and 2c) is very similar compared to configuration 1. The gradient run has no measurable impact on repeatability. The resulting standard deviation constantly remains below 1 %. The same is more or less true for the isocratic experiment.

CONFIGURATION 2 **1200 Series RRLC system**	**1100 Series Configuration 2B**	**1100 Series Configuration 2B**
Binary pump SL	Binary pump	Binary pump
Autosampler SL	Standard autosampler	Thermostatted standard autosampler
Column compartment SL	Column compartment	Column compartment
DAD/MWD SL	DAD/MWD	DAD

Table 2a
Configuration with standard autosampler.

GRADIENT **Chlorpheniramin**	**1200 RRLC**	**Configuration 2A**	**Deviation**	**Configuration 2B**	**Deviation**
RT [min]	5,05 ± 0,1 %	5,02 ± 0,0 %	**0,7 %**	5,14 ± 0,1 %	**1,8 %**
Peak width [min]	0,07 ± 0,5 %	0,06 ± 0,6 %	**1,2 %**	0,07 ± 0,5 %	**1,1 %**
Resolution	1,76 ± 0,5 %	1,87 ± 0,7 %	**6,2 %**	1,81 ± 0,4 %	**2,5 %**

Table 2b
The gradient run produced these repeatability results for chlorpheniramin, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

ISOCRATIC **Theophylline**	**1200 RRLC**	**Configuration 2A**	**Deviation**	**Configuration 2B**	**Deviation**
RT [min]	2,55 ± 0,2 %	2,55 ± 0,5 %	**0,3 %**	2,58 ± 0,1 %	**1,5 %**
Peak width [min]	0,08 ± 0,7 %	0,08 ± 2,8 %	**0,7 %**	0,08 ± 0,5 %	**0,5 %**
Resolution	5,34 ± 0,5 %	5,26 ± 1,2 %	**1,5 %**	5,36 ± 0,4 %	**0,4 %**

Table 2c
The isocratic run produced these repeatability results for theophylline, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

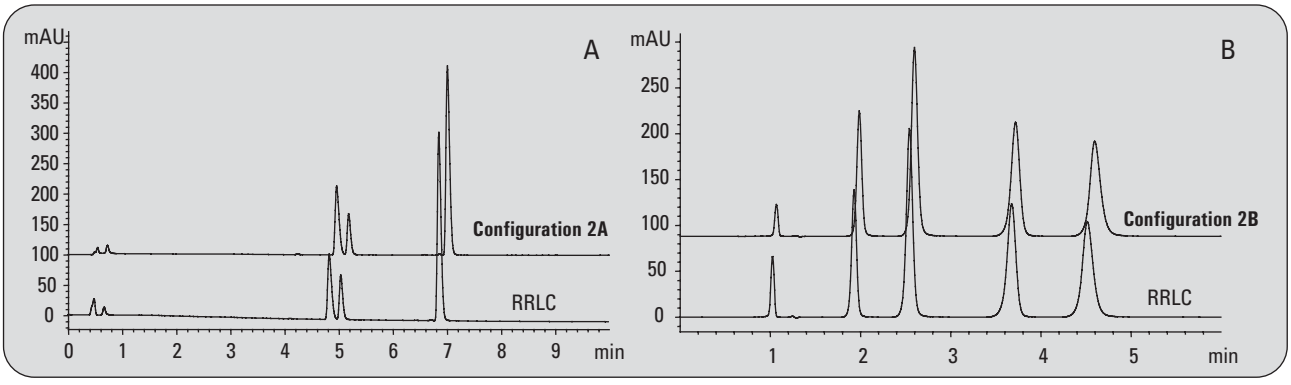

Figure 3
A) Comparison of performance, gradient run.
B) Comparison of performance, isocratic run.

In configuration 3 (table 3a) the 1100 Series quaternary pump was used instead of the 1100 Series binary pump, which allows a comparison of the 1200 Series binary pump with the 1100 Series quaternary pump. In addition to the different mixing principles, the quaternary pump has a higher delay volume. Therefore, the retention time, peak area, peak width and resolution differences are higher than for configurations 1 and 2 but still below 5 %, as shown in tables 3b and 3c. Once again the repeatability measurements constantly exhibit standard deviations below 1 % for all three characteristics.

CONFIGURATION 3 **1200 Series RRLC system**	**1100 Series Configuration 3A**	**1100 Series Configuration 3B**
Binary pump SL	Quaternary pump	Quaternary pump
Autosampler SL	Well-plate autosampler	Thermostatted well-plate autosampler
Column compartment SL	Column compartment	Column compartment
DAD/MWD SL	DAD/MWD	DAD

Table 3a
Configuration with quaternary pump.

GRADIENT **Chlorpheniramin**	**1200 RRLC**	**Configuration 3A**	**Deviation**	**Configuration 3B**	**Deviation**
RT [min]	5,05 ± 0,1 %	4,96 ± 0,0 %	**1,8 %**	4,97 ± 0,0 %	**1,6 %**
Peak width [min]	0,07 ± 0,5 %	0,06 ± 0,6 %	**1,5 %**	0,06 ± 0,6 %	**1,1 %**
Resolution	1,76 ± 0,5 %	1,89 ± 0,7 %	**7,2 %**	1,85 ± 0,4 %	**4,9 %**

Table 3b
The gradient run produced these repeatability results for chlorpheniramin, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

ISOCRATIC **Theophylline**	**1200 RRLC**	**Configuration 3A**	**Deviation**	**Configuration 3B**	**Deviation**
RT [min]	2,55 ± 0,2 %	2,45 ± 0,1 %	**3,6 %**	2,44 ± 0,1 %	**4,0 %**
Peak width [min]	0,08 ± 0,7 %	0,07 ± 0,8 %	**3,2 %**	0,07 ± 0,0 %	**4,1 %**
Resolution	5,34 ± 0,5 %	5,13 ± 0,6 %	**4,0 %**	5,08 ± 0,3 %	**4,8 %**

Table 3c
The isocratic run produced these repeatability results for theophylline, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

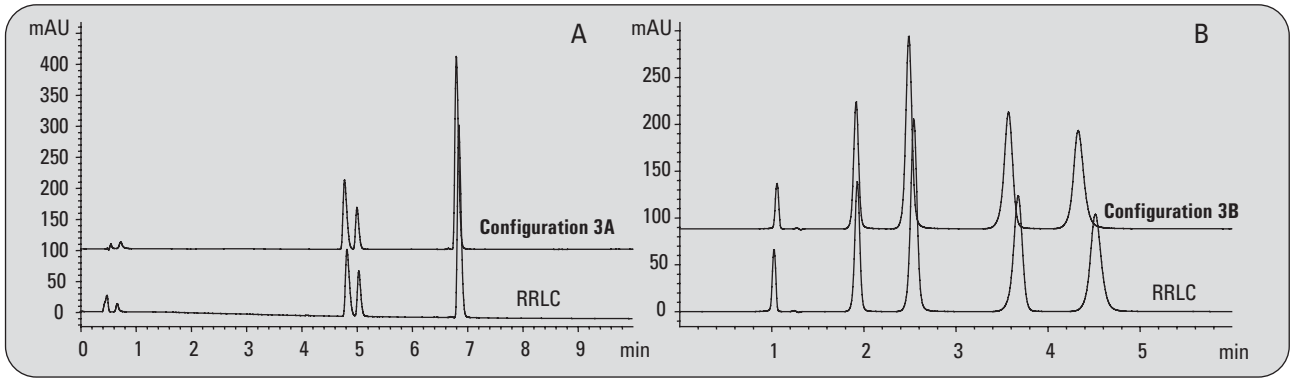

Figure 4
A) Comparison of performance, gradient run.
B) Comparison of performance, isocratic run.

In configuration 4 (table 4a) a VWD SL was used in the 1200 Series RRLC system and a VWD was used in the 1100 Series system. As both detectors have the same flow cell, the differences in retention time, peak area, peak width and resolution are less than 5 %, as shown in tables 4b and 4c. No major impact on repeatability could be determined.

CONFIGURATION 4 **1200 Series RRLC system**	**1100 Series Configuration 4A**	**1100 Series Configuration 4B**
Binary pump SL	Binary pump	Binary pump
Autosampler SL	Well-plate autosampler	Thermostatted well-plate autosampler
Column compartment SL	Column compartment	Column compartment
VWD SL	VWD	VWD

Table 4a
Configuration with VWD SL and VWD.

GRADIENT **Chlorpheniramin**	**1200 RRLC**	**Configuration 4A**	**Deviation**	**Configuration 4B**	**Deviation**
RT [min]	5,06 ± 0,1 %	5,10 ± 0,1 %	**0,9 %**	5,12 ± 0,0 %	**1,3 %**
Peak width [min]	0,07 ± 0,6 %	0,07 ± 0,0 %	**1,6 %**	0,07 ± 0,4 %	**1,8 %**
Resolution	1,66 ± 0,4 %	1,73 ± 0,4 %	**4,3 %**	1,74 ± 0,4 %	**4,9 %**

Table 4b
The gradient run produced these repeatability results for chlorpheniramin, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

ISOCRATIC **Theophylline**	**1200 RRLC**	**Configuration 4A**	**Deviation**	**Configuration 4B**	**Deviation**
RT [min]	2,62 ± 0,2 %	2,65 ± 0,1 %	**1,2 %**	2,64 ± 0,7 %	**0,8 %**
Peak width [min]	0,08 ± 1,0 %	0,08 ± 0,9 %	**1,0 %**	0,08 ± 0,9 %	**0,2 %**
Resolution	5,22 ± 0,3 %	5,22 ± 0,6 %	**0,0 %**	5,21 ± 1,0 %	**0,1 %**

Table 4c
The isocratic run produced these repeatability results for theophylline, (± relative standard deviation in percent). Characteristic differences between the 1200 Series system compared to the 1100 Series systems are listed as deviation and are expressed in percent.

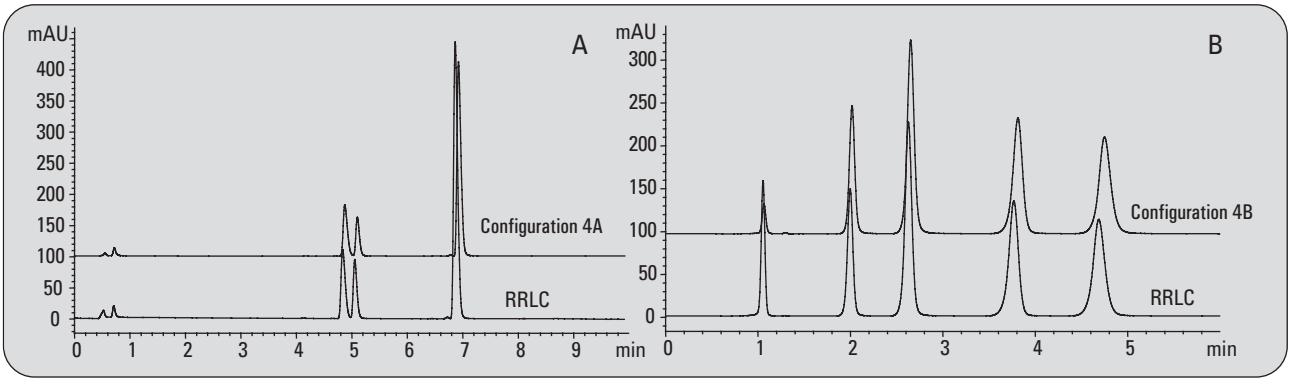

Figure 5
A) Comparison of performance, gradient run.
B) Comparison of performance, isocratic run.

Conclusion

Based on the results from the experiments performed on the Agilent 1200 Series RRLC system and on various configurations of an 1100 Series system, the following conclusions were drawn regarding the transfer of methods to the 1200 Series RRLC system:

1. Generally no specific impact on the repeatability was observed. As a conclusion, we do not expect any significant effect on other experiments under similar conditions.
2. Compared to an 1100 Series system, a difference of less than 3 % in retention time, peak area, peak width and resolution can be expected from a 1200 Series RRLC system running the same method if the 1100 Series system is equipped with
   - a binary pump,
   - a well-plate or standard autosampler,
   - a thermostatted column compartment, and
   - the same type of detector (VWD or DAD/MWD).
3. If the 1100 Series system contains a quaternary pump instead of a binary pump, the differences in peak area, retention time, peak width and resolution will increase to about 5 %.
4. If a thermostatted well-plate or thermostatted standard autosampler is used in the 1100 Series system, a thermostatted autosampler should also be used in the 1200 Series RRLC system to retain the same characteristics.

Further considerations should be made with respect to the column diameter. The experiments in this study were performed using the most widely used columns at present, having inner diameters of 4 and 4.6 mm and at most common flow rates of 1 mL/min. Higher differences regarding peak area, retention time, peak width and resolution can be expected when working with lower flow rates or on columns with smaller inner diameter.

The extent of revalidation necessary to transfer methods from an 1100 Series system to a 1200 Series RRLC system still has to be determined on a case-by-case basis. In principle, if the method was validated with a broad enough scope which tolerates differences in a range of 3 – 5 % for the described parameters, revalidation activities can be reduced to a minimum. Finally, a sound decision has to be made based on own experiments using the respective equipment and method.

It is important to note that the standard capillaries, as defined in the user manuals of the individual modules, must be used according to the system configuration in order to achieve the results described in this Technical Note.

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