Evaluation of linear and step gradient performance and retention time precision of the Agilent 1200 Series Rapid Resolution LC system

Technical Note

Introduction

A main performance criterion of any LC system is precision of retention times, which is influenced primarily by the pumping device. It is important that the selected flow is delivered precisely and that for gradient operation the mobile phases are mixed reliably over the complete gradient range. In this Technical Note the precision of retention times for gradient and isocratic applications is evaluated. Further, based on tracer experiments, it is demonstrated that linear and stepwise gradients are delivered with excellent accuracy and precision. The experiments also show that the variability of the delay volume meets the demands of applications using narrow-bore columns as well as standard-bore columns.

Experimental

Equipment

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

- Agilent 1200 Series binary pump SL with vacuum degasser for applications using 1.8-µm 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 lengths, packed with 1.8-µm particles

Pump design

The Agilent 1200 Series binary pump SL is designed to meet the demands of high throughput and highest performance for optimum resolution and lowest pump ripple for a wide range of applications on narrow or standard-bore columns. Figure 1 shows a schematic of the 1200 Series binary pump SL. The delay volume can be configured according to the application needs. The standard configuration is best suited for highest performance in terms of noise and resolution. In this standard configuration the mixer and damper are in the flow path and the delay volume is between 600 and 800 µL, depending on the pressure. This configuration is suited for applications where lowest noise and highest resolution for optimum quantification is needed. For applications where a low delay volume is needed the damper and the mixer can be switched out of the flow path, resulting in a delay volume of 120 µL. This configuration is best suited for application where speed and lowest peak dispersion are needed. To enable these changes the pressure transducer is separated from the damper. Both pump heads have an additional damping coil each of 500 µL volume and an additional outlet valve. This does not add delay volume during gradient analysis because mixing occurs later in the flow path. The pump can be used up to pressures of 600 bars, providing for the use of sub-2-micron particle columns up to 150-mm length. In the standard delay volume configuration the mixer and damper are in the flow path. To change to the low delay volume configuration, the flexible 0.17-mm id capillary between the mixer and the upper position of the pressure sensor is disconnected. Then, the capillary originally attached to the mixer is connected to the pressure sensor. The damper and mixer are now not in the flow path. Degassing is recommended for all application ranges to ensure optimum performance. The Agilent 1200 Series micro degasser SL has 4 channels and an internal volume of 1 mL. Each channel degasses the solvents continuously. The following sections discuss the performance of both pump configurations; with and without mixer and damper in the flow path. The configuration with mixer and damper in the flow path delivers optimum resolution and lowest pump ripple and is best suited for the analysis of complex samples where highest

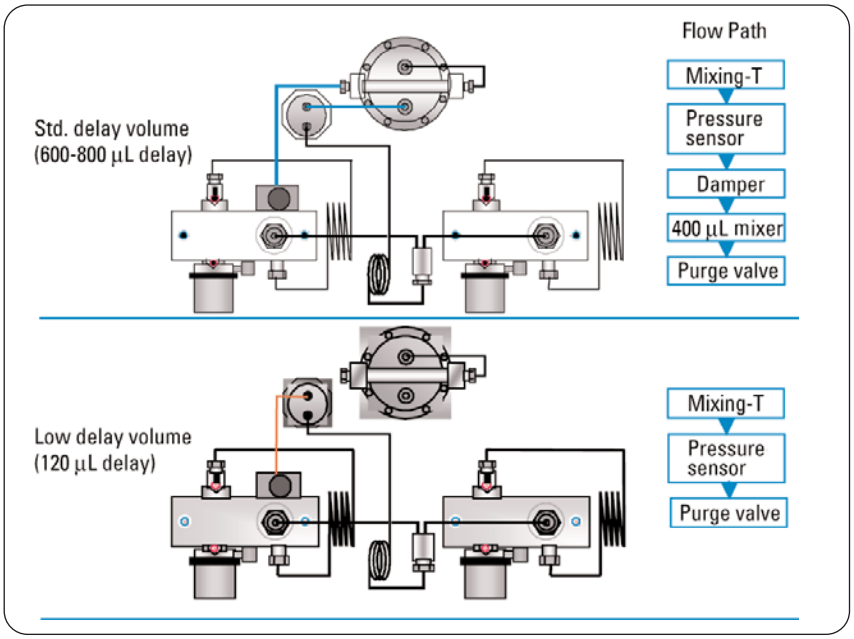

Figure 1
Standard and low delay volume configurations of the Agilent 1200 Series binary pump SL.

resolution and lowest system noise is needed. The configuration without damper and mixer in the flow path is best suited for LC/MS or LC/UV using 2.1-mm ID columns.

Retention time precision of Agilent 1200 Rapid Resolution LC system for gradient LC analysis

Retention time precision was evaluated for both pump configurations under gradient and isocratic conditions using standard bore and narrow bore columns. In the first example the standard delay configuration was tested using a standard bore column.A “phenone mix“ was analyzed using gradient conditions from 35 to 95 %. A 100 x 4.6 mm column and a flow rate of 1.5 mL/min were selected. These conditions are close to conditions used in conventional LC analysis. Figure 2 shows 10 runs overlaid, demonstrating the retention time precision obtained for this application. The run time was 6 min. The evaluation of the retention time precision was found to be less than 0.07 % RSD.

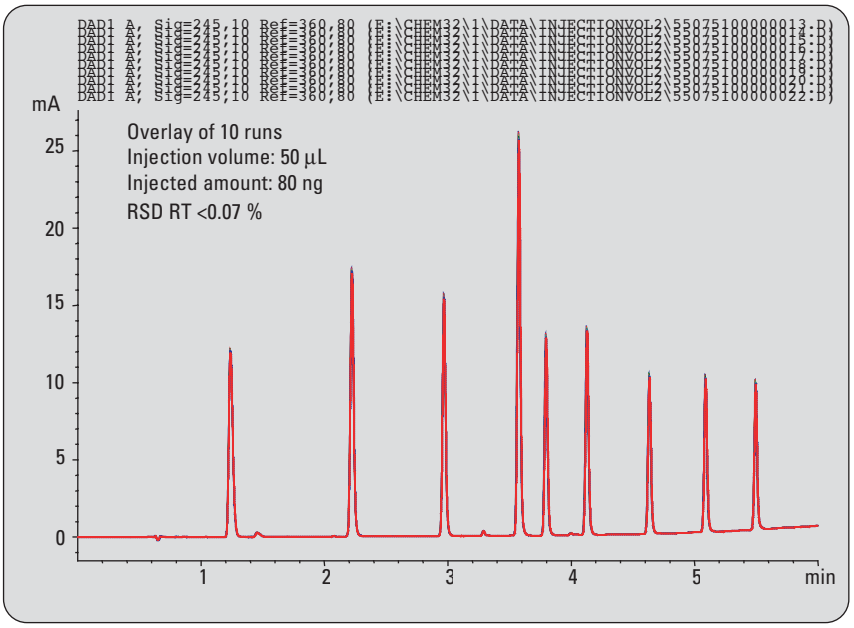

Figure 2
Overlay of 10 chromatograms.

Chromatographic conditions:

Test sample:	Set of 9 compounds, 100 ng/uL each, dissolved in water/ACN (65/35)	
	1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone (200 ng/mL),	
	5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone,	
	9. Octanophenone	
Column:	100 x 4.6 mm ZORBAX SB C-18, 1.8 µm for 600 bar operation	
Pump:	Solvent:	A = Water, B = ACN
	Gradient:	35 to 95 %B in 5 min, hold at 95 %B for 1 min
	Stop Time:	6 min
	Post Time:	2 min
	Flow Rate:	1.5 mL/min
Autosampler:	Injection volume: 50 µL	
	Wash 10 s for exterior of needle	
Thermostatted column compartment:		
	Temperature: 50 °C	
Detector:	13 µL cell	
	20-Hz data acquisition	
	Peak width = <0.01 min	
	Slit:	8 nm
	Signal:	245/10 nm, ref 360/80 nm

Retention time precision of Agilent 1200 Rapid Resolution LC system using the low-delay volume configuration

In the previous section the retention time precision of the standard delay configuration was evaluated. In this section the retention time precision of the low volume delay configuration will be evaluated. The analyses were run at high and low flow rates as well as at high and low temperatures. For all low flow rate applications the relative standard deviation for the retention times was typically less than 0.07 %. For high flow rate and ultra-fast applications the relative standard deviation for the retention times was typically less than 0.14 %, see figure 3 and also literature reference 1.

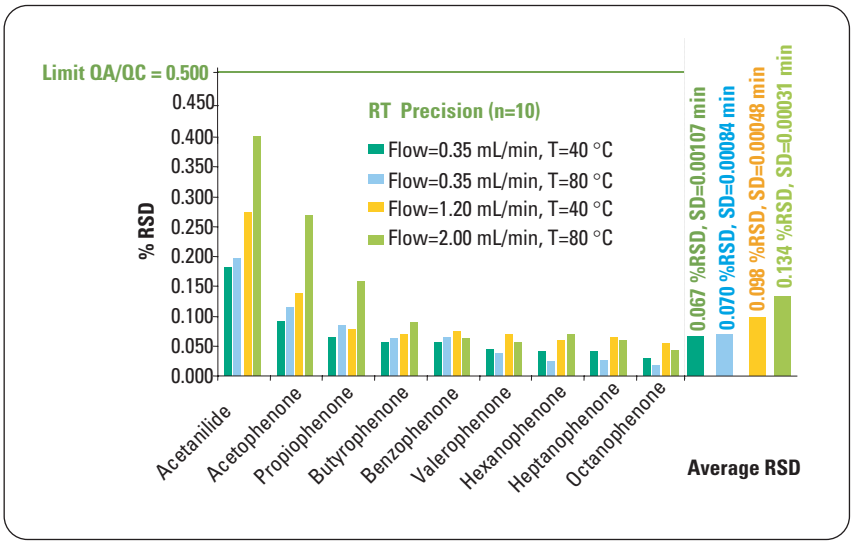

Figure 3
Statistical evaluation of the retention time precision for the repeated analysis of a nine-compound mixture under various conditions using the low delay configuration of the Agilent 1200 Series Rapid Resolution LC system.

Chromatographic conditions:

Solvent:	A = Water, B = ACN
Temp.:	40 °C, 80 °C
Flow:	0.35 mL/min, 1.20 mL/min, 2.0 mL/min
Gradient:	0.00 min 35 %B 2.60 min 95%B 3.20 min 95%B 3.21 min 35%B Time values for F = 0.35 mL/min. For all other flow rates times are scaled so that (time x flow) = 0.90 mL
Stop time:	3.20 min
Post time:	2.00 min
Wavelength:	245 nm (8), Ref. 450 nm (100)
Slit:	8 nm
Peak width:	> 0.0025 min (0.05 s response time), 80 Hz
Spectra:	All, 190-500 nm, bandwidth = 1 nm
Injection vol.:	1.0 µL
Injector:	Overlapped injection, Automatic delay volume reduction, Sample flush out factor = 10 Needle wash = 5 s
Sample:	"Phenone mix" (0.1 µg/µL each, 0.2 µg/µL of Butyrophenone)

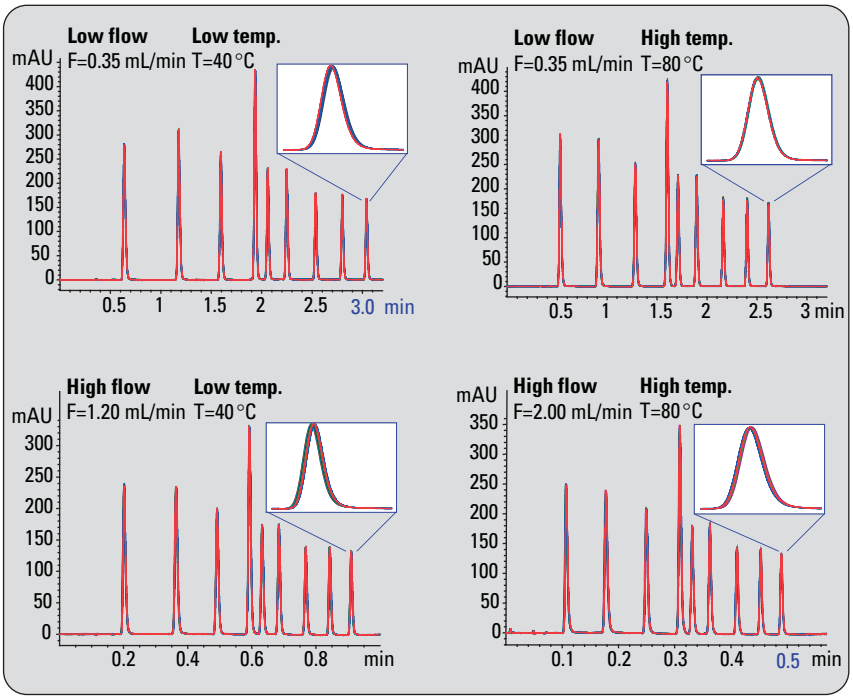

Figure 4
Overlaid chromatograms of the repeated analysis of a 9-compound mixture under various conditions.

Chromatographic conditions:

Sample:	Isocratic standard sample, 1. dimethylphthalate 0.15 wt%, 2. diethylphthalate 0.15 wt%, 3. biphenyl 0.01 wt%, 4. o-terphenyl 0.03 wt%
Column:	2.1 x 100 mm, ZORBAX SB C-18, 1.8 µm
Mobile phase:	Water/acetonitrile = 30/70
Injection vol.:	3 µL
Flow rate:	0.5 mL/min

Retention time precision of Agilent 1200 Rapid Resolution LC system for isocratic LC analysis

In this example isocratic conditions were selected, see conditions in figure 5. A narrow bore column was chosen and the flow rate was set to 0.5 mL/min. In figure 5 an overlay of 10 runs is shown for the analysis of test mixture. Typically the relative standard deviation for this type of application is less than 0.07 % for retention times.

Delay volume, system ripple, precision and accuracy of gradients

To evaluate pump performance tracer experiments are a frequently used tool to evaluate the system ripple at different gradient mixtures. The delay volume and the accuracy and the precision of gradients are also evaluated using step gradients and linear gradients. Figure 6 shows a step gradient from 0 to 100 % in 10 % steps using the Agilent 1200 Series Rapid Resolution LC system configured for standard delay volume.

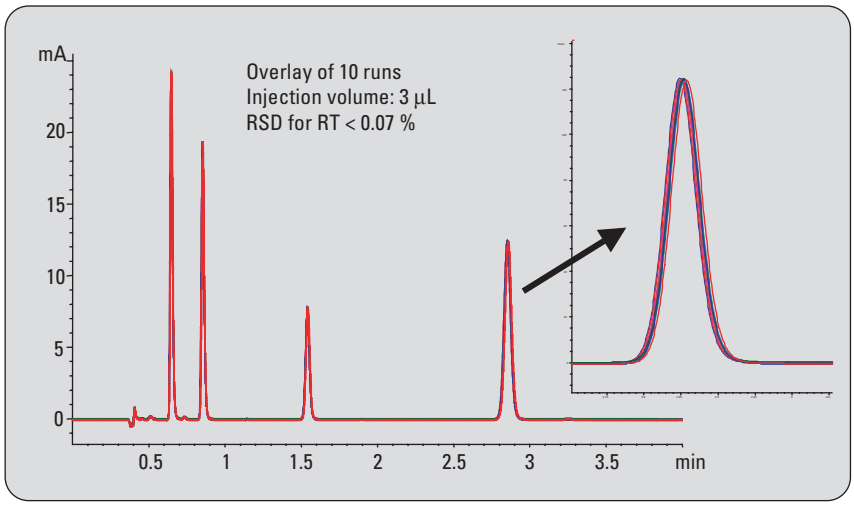

Figure 5
Precision of retention times on 2.1-mm column, isocratic elution, overlay of 10 runs, RSD of RT less than 0.07 %.

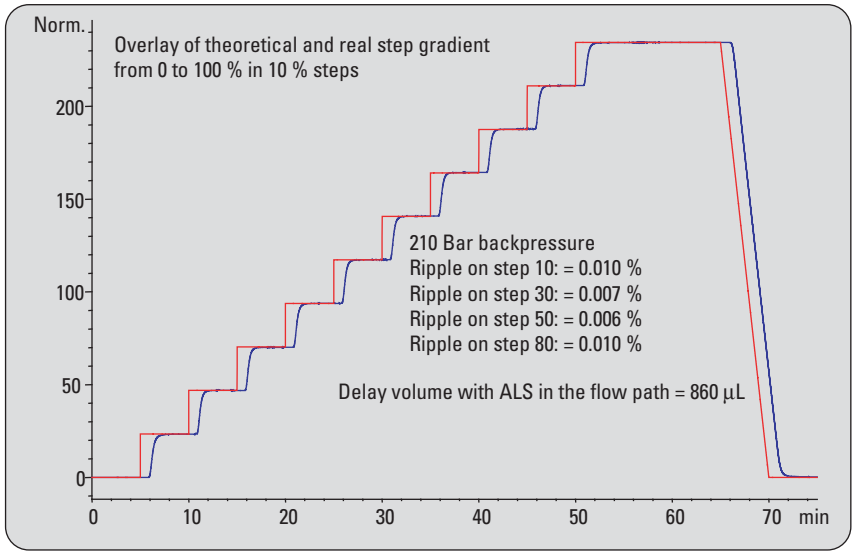

Figure 6
Accuracy, delay volume and ripple using the standard delay configuration of the Agilent 1200 Series Rapid Resolution LC system at 210 bar backpressure, 0 to 100 %B gradient in 10 % steps.

Chromatographic conditions:

Column:	Restriction capillaries
Mobile phases:	A=water, B=water/0.5 % acetone
Flow rate:	1 mL/min
Step gradients:	0 %B to 10 %B in 1 % steps, each step held for 5 min 0 %B to 100 %B in 10 % steps, each step held for 5 min
Linear gradient:	100 % to 0 % in 5 min
Temperature:	36 °C
Detection:	Signal 265/20 nm, reference 360/100 nm Slit 4 nm Peak width 0.01 min 13 µL cell

With the autosampler in the flow path the delay volume is about 860 µL. The system ripple is less than or equal to 0.01 % related to the 100 % step. The overlay with theoretical step gradient shows excellent agreement with the real step gradient with regard to the step heights. Figure 7 shows an overlay of 3 step gradients using the standard delay volume configuration of the Agilent 1200 Series Rapid Resolution LC system. The steps are 1 % from 0 to 10 %.
The precision for the 3-step gradient runs is excellent and the system ripple for the 1 % steps is less than or equal to 0.011 % related to the 100 % step. Figure 8 shows an overlay of 3 linear gradients using the low delay volume configuration of the Agilent 1200 Series Rapid Resolution LC system. The linear gradient was run from 100 % to 0 % tracer. The overlay shows that precision for a linear gradient is also excellent for the low delay volume configuration. The delay volume in this configuration is about 420 µL with autosampler in the flow path. If the autosampler is taken out of the flow path the delay volume is about 120 µL. The system ripple with the autosampler in the flow path is typically less than 0.05 % related to the 100 % step.

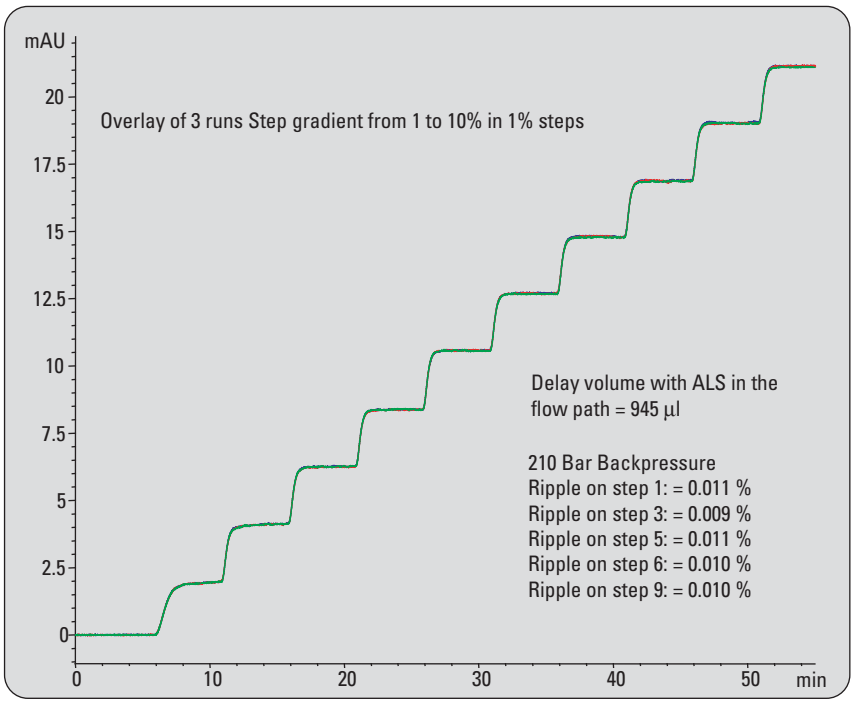

Figure 7
Precision and ripple using the standard delay configuration of the Agilent 1200 Series Rapid Resolution LC system at 210 bar backpressure, 0 to 10 % in 1 % steps.

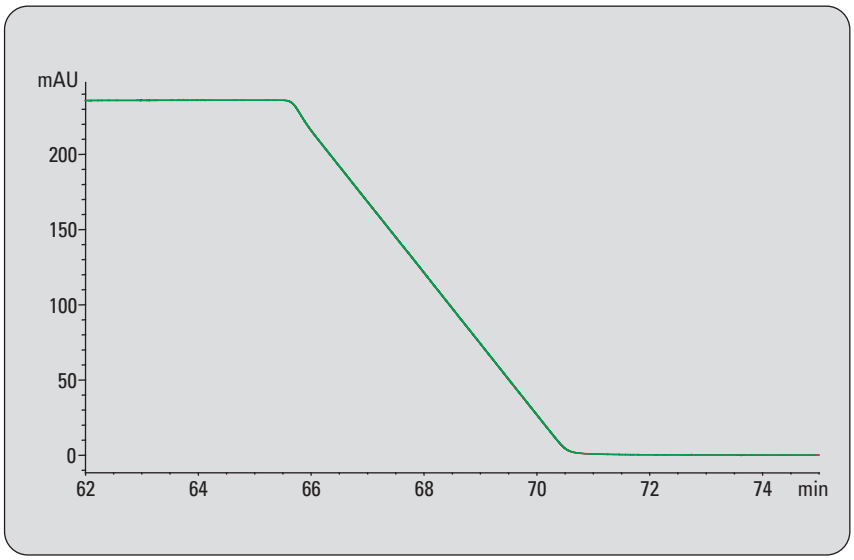

Figure 8
Precision using the low delay volume configuration of the Agilent 1200 Series Rapid Resolution LC system at 200 bar backpressure, linear gradient from 100 to 0 %, 3 overlays.

Conclusion

The data presented in this note show that the precision of retention time of the Agilent 1200 Series binary pump SL pump is excellent for a wide range of LC applications using either narrow-bore or standard-bore columns. Typically a precision for retention times of less than 0.07 % relative standard deviation can be expected for isocratic and gradient applications using the standard delay volume configuration of the pump. For the low delay volume configuration the retention time precision is typically less than 0.07 % relative standard deviation for more conventional flow rates and is typically less than 0.14 % for ultra-fast applications with run times less than 1 min.

Accuracy and precision of linear and step gradients are excellent. Ripple for the standard delay volume configuration is typically less than or equal to 0.01 %. The low delay volume configuration has a system ripple typically less than 0.05 %. The delay volume variability supports the needs for low delay volume applications as well as the demands for delay volume needed for more conventional validated methods. The standard delay volume configuration with the autosampler in the flow path has a delay volume of 860 µL at 200 bar backpressure. The low delay volume configuration has a delay volume of 120 µL when the autosampler is switched out of the flow path.

References

1.
"Performance characteristics of the Agilent 1200 Series LC system", *Agilent Application Note, Publication Number 5989-4489EN*, **2006.**

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