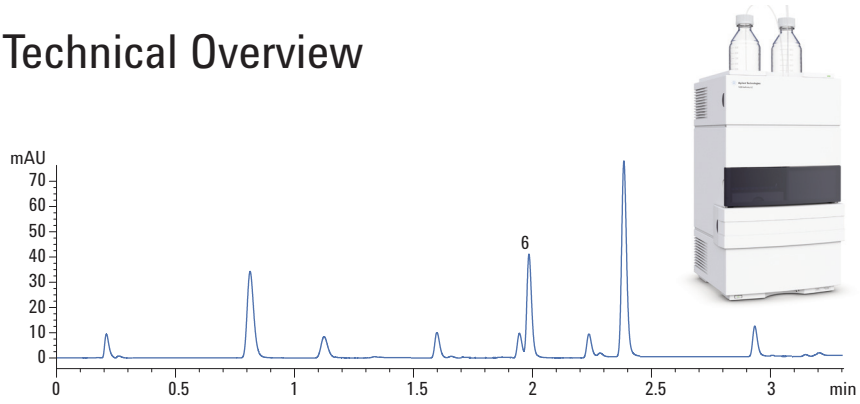
Performance Characteristics of the Agilent 1220 Infinity Gradient LC system

An integrated LC system for conventional LC and UHPLC

Technical Overview



Introduction

The Agilent 1220 Infinity Gradient LC is a liquid chromatography (LC) system for routine standard analysis. Due to its extraordinary pressure range up to 600 bar, the system can also perform UHPLC applications. It is an integrated LC system consisting of a binary low pressure mixing ('dual gradient') pump, autosampler, column compartment and variable wavelength detector (VWD). The dual gradient pump has a flow range from 0.2 up to10 mL/min (5 mL at 600 bar, 10 mL at 200 bar), low pressure mixing and an integrated degasser. The VWD detector features 80 Hz data acquisition rate and a wavelength range from 190 nm up to 600 nm. The autosampler can operate up to 600 bar, with an injection volume range from 0.1 to 100 µL and a capacity of 100 2 mL vials. The column oven holds one 25 cm column and the maximum temperature is 60 °C.

In this Technical Overview the following parameters were tested on the Agilent 1220 Infinity Gradient LC:

The pump was tested for:

- Precision of retention time
- Performance of step gradients

The autosampler was tested for

- Precision of areas
- Carryover
- Injection volume linearity

The variable wavelength detector was tested for:

- Noise and drift
- Linearity

Method transfer from conventional to UHPLC is another important application which will be demonstrated.

Agilent Technologies

Experimental

The Agilent 1220 Infinity LC Gradient System (G4290B) was equipped with dual gradient pump, autosampler, column compartment and the variable wavelength detector.

Pump performance–Precision of retention times

Optimum retention time precision depends mainly on:

- Pump performance, the most important issue
- Equilibration status of the columns
- Equilibration status of the complete system
- Degassing of the solvent
- Temperature stability of the column compartment

The most important parameter is the pump performance itself, however, other parameters can influence precision of retention times. For example, if a solvent was changed the column needs at least ten column volumes for proper equilibration. If gradients are applied, at least five column volumes are needed to equilibrate the column to the start conditions. Furthermore, if the column compartment temperature was changed, for example from 30 °C to 60 °C, it requires approximately 20 minutes until the column is equilibrated to the new temperature. Proper degassing influences the precision positively.

In this Technical Overview, retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3 mm internal diameter (id) columns. The relative standard deviation (RSD) for retention times is typically < 0.2% RSD for gradient analysis. In Figure 1, an example of an isocratic application is shown. Here the flow precision is typically < 0.07% RSD for retention times.

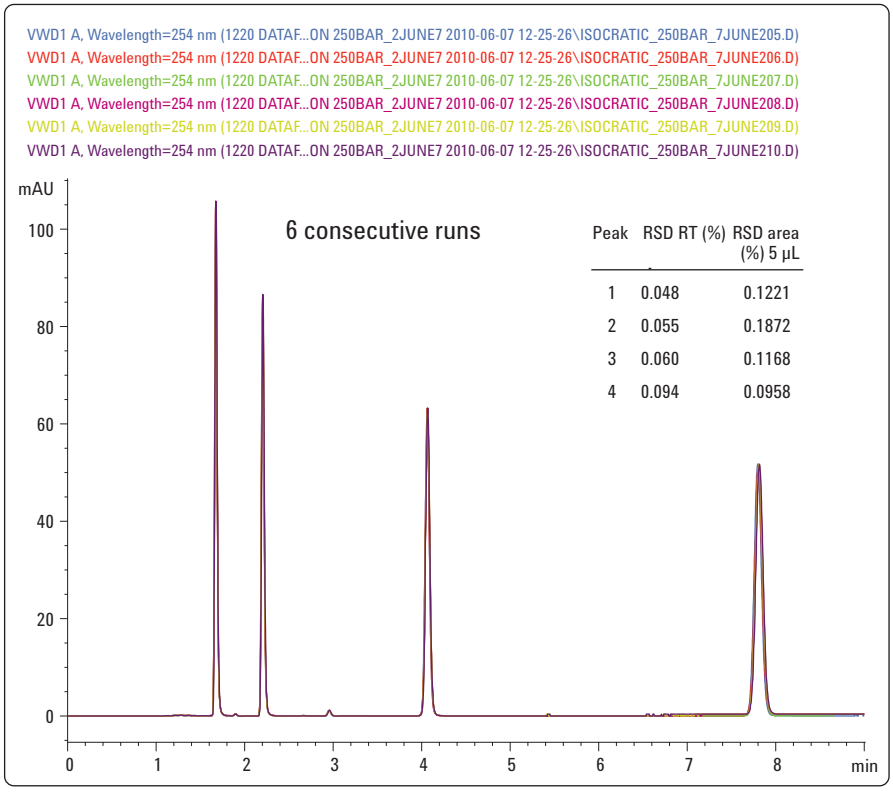


Figure 1
Retention time and area precision for an isocratic run run with dynamic mixing.

Sample	Isocratic test sample (p/n 01080-68707)
Column	Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 1.8 µm (PN 959994-902), 245 bar
Mobile phase	A = Water B = Acetonitrile
Isocratic	30/70 A/B
Flow rate	1.2 mL/min
Stop time	9 min
Injection volume	5 µL , draw speed 200 µL/min
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width:	PW 0.05 min (10 Hz)

In Figure 2 an example for a gradient run is shown. The retention time precision is < 0.045%RSD, except for the first peak

In Figure 3 an example for a fast gradient run at 563 bar is shown. In this application Poroshell columns were used. The retention time precision is < 0.15%RSD.

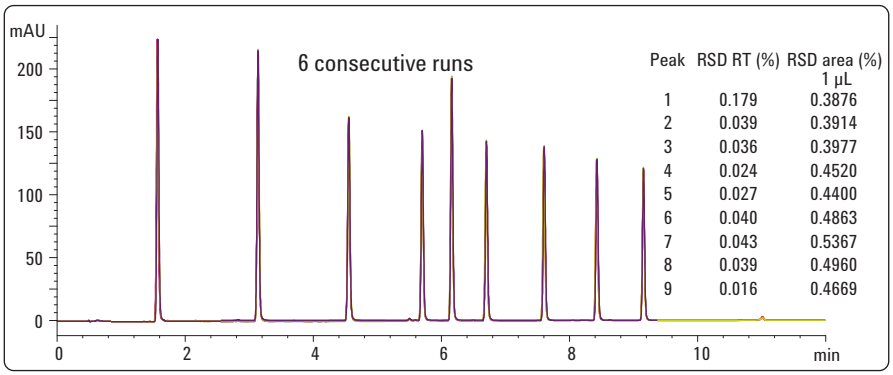


Figure 2
Precisions for a gradient run at 445 bar with a RT precision < 0.045% RSD except for the first peak.

Sample	RRLC checkout sample (p/n 5188-6529)
Column	Agilent ZORBAX Eclipse Plus C18 3 × 100 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	20% B to 95% B in 10 min
Flow rate	1 mL/min
Stop time	12 min
Post time	5 min
Injection volume	1 µL
Column temperature	40 °C
VWD	245 nm
Flow cell	10 mm
Peak width	> 0.05 min (10 Hz)

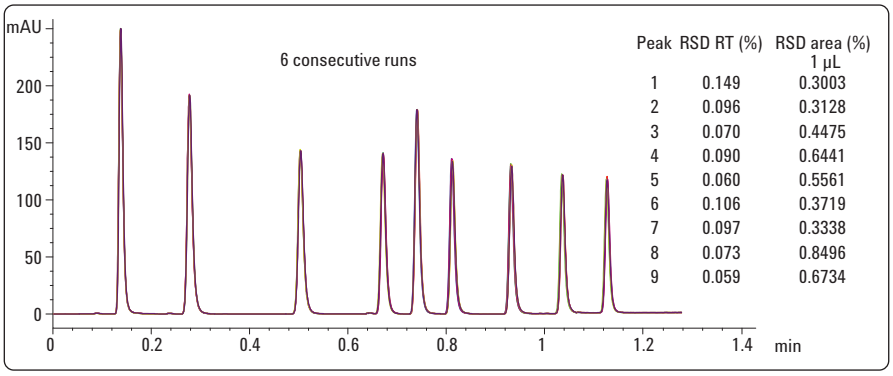


Figure 3
Retention time and area precision at 563 bar for a fast gradient application. RSD RT is < 0.15.

Sample	RRLC checkout sample (p/n 5188-6529)
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	30% B to 95% B in 1 min
Flow rate	3.5 mL/min
Stop time	1.5 min
Post time	1 min
Injection volume	1 µL
Column temperature	40 °C
VWD	245 nm
Flow cell	10 mm
Peak width	PW > 0.0125 min (40 Hz)

Performance of step gradient

Tracer experiments are also frequently used to verify the solvent mixing ripple at different gradient mixtures, to evaluate pump performance. The delay volume, the accuracy and precision of gradients are also evaluated using step gradients. Figure 4 shows a step gradient from 0 to 100% in 10% steps. Caffeine was selected as the tracer compound. Acetone is not ideal for testing step gradient performance because acetone is too easily removed in the degasser at low flow rates. For testing the step gradient performance of the Agilent 1120 Infinity LC, we recommend using non-volatile compounds.

The performance results are :

- Ripple on 10% step = 0.03%
- Ripple on 50% step = 0.08%
- Ripple on 90% step = 0.08%
- Precision of step height for 50% step = <0.1%RSD for 3 runs
- Delay volume: 860 µL

Injector performance–Area precision

Precise injection is mandatory for good quantitative results in liquid chromatography. The Agilent 1220 injector can inject precisely over an injection range from 0.5 up to 100 µL. Examples are given in Figures 1 to 3 . In Figure 1 the Area precision is < 0.19% RSD for 5 µL injection volume. Figure 2 shows an example of a conventional gradient. The area precision for 1 µL is < 0.54% RSD. The area precision for 1 µL is <0.85% RSD for a fast gradient run as shown in Figure 3.

The injector settings are very important for optimum precision of areas. For example, if highest precision is needed the draw speed of the injector should be set to lower values, especially if large volume or viscous sample are injected.

Carryover

Carryover was tested using the built in Agilent 1220 autosampler. The injection draw speed was set to 20 µL/min and an exterior needle wash was used. The carryover (Figure 5) was found to be < 0.031% for the conditions used. After a 500 ng sample injection, unadulterated solvent was injected.

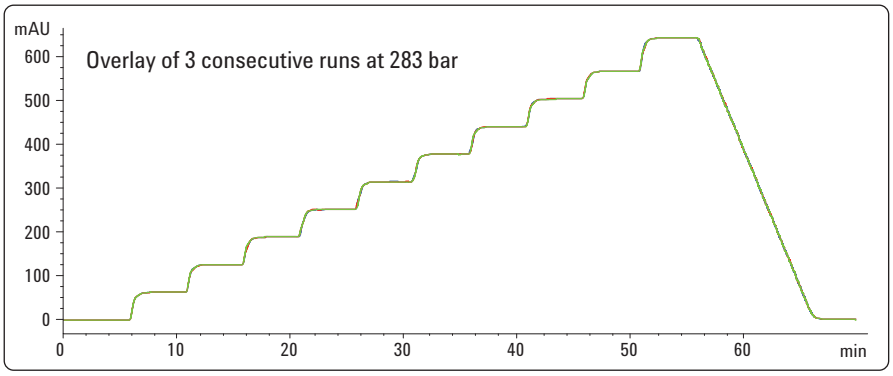


Figure 4
Overlay of 3 step gradients.

Column	Restriction capillary with 283 bar backpressure
Mobile phase	A = Water +20% Isopropanol
	B = Water + 20% Isopropanol +10 mg/L Caffeine
Step gradient	from 0% to 100% B in 10% steps
Flow rate	1 mL/min
Stop time	70 min
Post time	5 min
Column temperature	36 °C
VWD	273 nm
Flow cell	10 mm
Peak width	> 0.025 min (20 Hz)

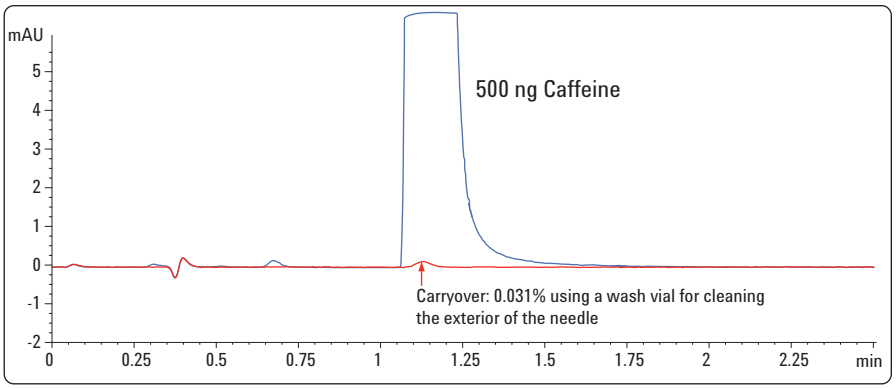


Figure 5
Carryover of 500 ng injection of Caffeine is 0.031%.

Sample	Caffeine 500 ng/µL
Column	Agilent Poroshell 120 EC C-18 3 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Isocratic	10% B
Flow rate	0.8 mL/min
Stop time	2.5 min
Injection volume	1 µL , draw speed 20 µL/min, wash vial in position 41
Column temperature	30 °C
VWD	273 nm
Flow cell	10 mm
Peak width	PW > 0.025 min (20 Hz)

Injection volume linearity

Injection volume linearity was tested using Primidone standards. All injection volumes contained 781.26 ng of Primidone. The injection volume varied but the injected amount was the same, (Figure 6). The peak heights and areas are expected to be the same for all injection volumes. The experiments show that all areas were within 0.82% RSD over the complete injection volume range of 0.8 to 100 µL. Each injection volume was injected 3 times and the resulting 24 runs were evaluated for area precision.

Prepare an accurate dilution series to obtain good linearity. One way is to dilute large volumes, for example starting with one liter. If only small volume should be diluted special care has to be taken: The pipettes should be calibrated, and the same pipette should be used for the complete dilution series. Otherwise there is a big risk that a dilution error is measured rather than linearity.

Detector performance

Evaluation of baseline noise according to guidelines of the American Society for Testing and Materials (ASTM) and drift of the 10 mm and 60 mm path length cell.

ASTM noise and drift was evaluated using a restriction capillary instead of a column and water as the mobile phase. The variable wavelength detector was set to a four seconds response time. The resulting ASTM noise of the 10 mm path length cell was found to be ±2.2 µAU and the drift was1.2 mAU/h (Figure 7).

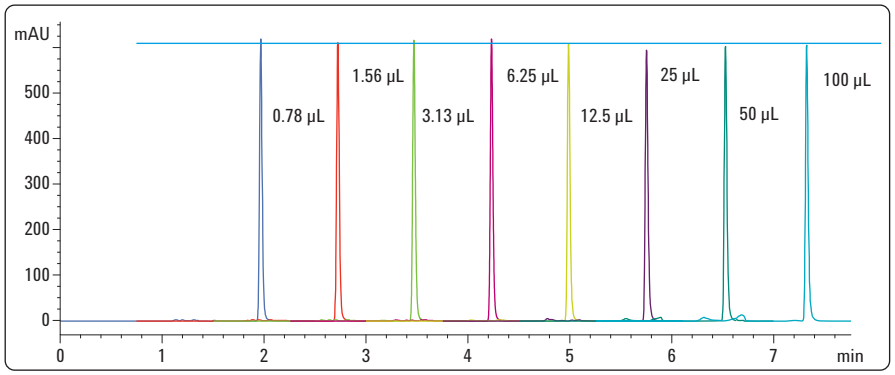


Figure 6
Injection volume linearity from 0.8 to 100 µL using Primidone as test compound.

Sample	Primidone 25 mg/25 mL, 7 times 1:2 diluted
Column	Agilent ZORBAX Eclipse Plus C18 150 × 4.6 mm,1.8 µm
Mobile phase	A = Water B = Acetonitrile
Isocratic	30% B
Flow rate	0.8 mL/min
Stop time	2.5 min
Injection volume	0.78 to 100 µL , draw speed 50 µL/min
Column temperature	40 °C
VWD	220 nm
Flow cell	10 mm
Peak width	PW > 0.025 min (20 Hz)

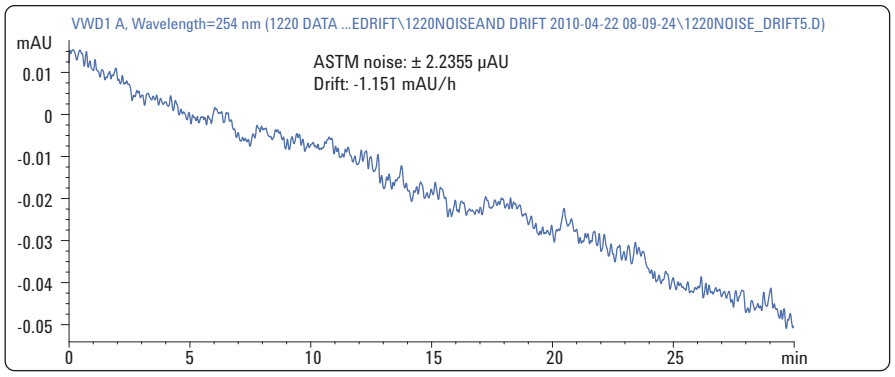


Figure 7
VWD noise and drift measurement.

Column	Restriction capillary with 42 bar backpressure
Mobile phase	A = Water isocratic
Flow rate	1 mL/min
Stop time	30 min
Column temperature	36 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.2 min (4 s response) (2.5 Hz)

Linearity for different caffeine concentrations

Linearity was tested using caffeine standards from 1.5 ng to 750 ng of injected amount. For this concentration range, very good linearity was obtained. The coefficient of correlation was 0.99996. The response factors were all within the 5% error range from 1.5 up to 750 ng (Figure 8).

Method transfer from conventional to UHPLC

In this experiment, columns of different pore size and length were employed to shorten analysis and optimize resolution (Figure 9).

It has been shown that the Agilent 1220 Infinity LC system is an instrument that can be used for conventional chromatography (Figure 9, blue trace) as well as UHPLC (other traces). It is possible to progress step by step to UHPLC and optimize for resolution (Figure 9, green trace) or for speed (pink trace).

Chromatographic conditions

Sample from Sigma Aldrich:

- Reversed Phase Test Mix (Order No.: 47641-U)
- 1 × 1 mL (uracil, phenol, n,n-diethyl-m-toluamide, toluene)
- HPLC Gradient System Diagnostic Mix (Order No.: 48271)
- 6 × 1 mL (phenol, methyl parabens, ethyl parabens, propyl parabens, butyl parabens, hepthyl parabens, uracil)

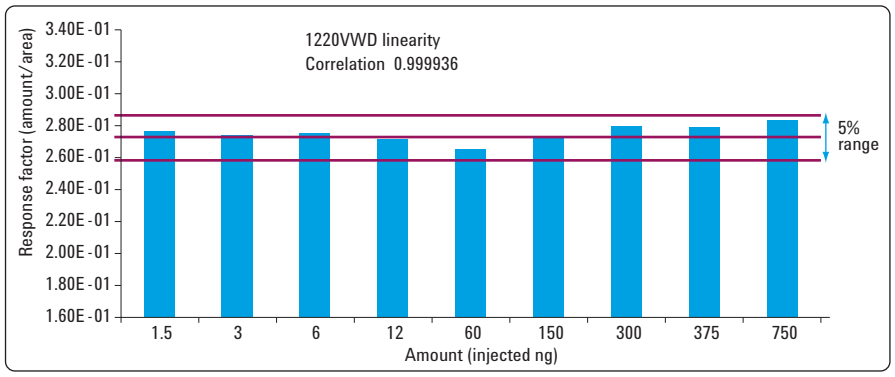


Figure 8
VWD linearity using Caffeine as sample compound.

Sample	Caffeine standards
Column	Agilent Poroshell 120 EC-C18, 3.0 × 50 mm, 2.7 µm
Mobile phase	A = Water
	B = Acetonitrile
Isocratic	10% B
Flow rate	0.8 mL/min
Stop time	1.5 min
Post time	1 min
Injection volume	3 µL
Column temperature	30 °C
VWD	273 nm
Flow cell	10 mm
Peak width	PW 0.025 min (20 Hz)

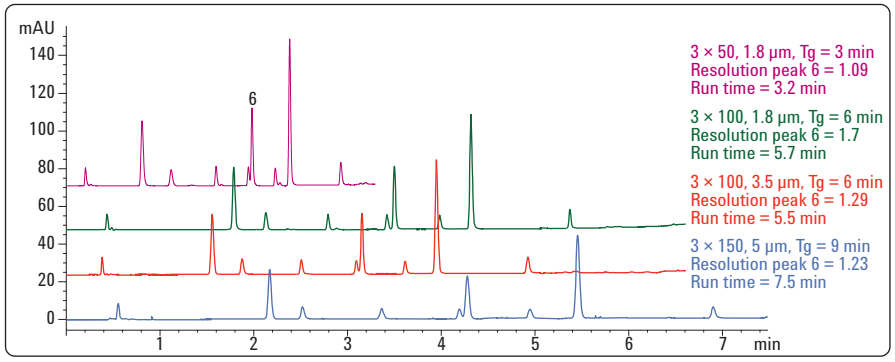


Figure 9
Optimization of resolution and speed by changing step by step from conventional to UHPLC.

Sample preparation
Dilute each sample to 5 mL with water/acetonitrile 1:1. Mix the two diluted samples 1:1

Column	Agilent ZORBAX Eclipse Plus C18, 3 x 50, 1.8 µm, 3 x 100, 1.8 µm, 3 x 100, 3.5 µm 3 x 150, 5 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient:	0 min 20 % B
	3 min, 6 min, 6 min, 9 min 100% B
Flow rate	1.2 mL/min
Stop time	3.2 min, 5.7 min, 5.5 min, 7.5 min
Injection volume	3 µL
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.005 min (80 Hz)

It is also possible to obtain better resolution in less time by further increasing the flow rate, (Figure 10).

Chromatographic conditions

Sample from Sigma Aldrich:

Reversed Phase Test Mix (Order No.: 47641-U)

1 × 1 mL (uracil, phenol, n,n-diethyl-m-toluamide, toluene)

HPLC Gradient System Diagnostic Mix (Order No.: 48271)

6 × 1 mL (phenol, methyl parabens, ethyl parabens, propyl parabens, butyl parabens, hepthyl parabens, uracil)

Sample preparation:

Dilute each sample to 5 mL with water/acetonitrile 1:1

Mix the two diluted samples 1:1

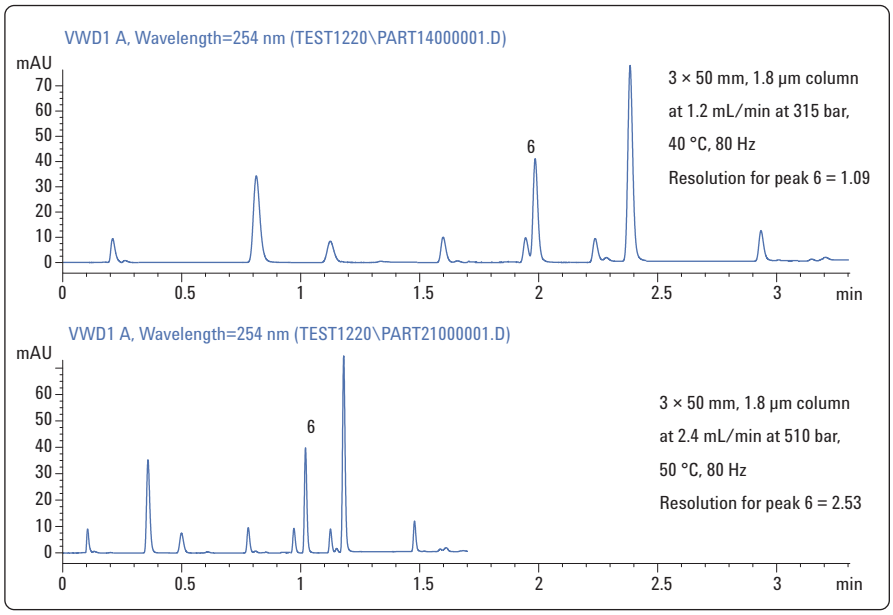


Figure 10
Improving speed and resolution by increasing the flow rate.

Column	Agilent ZORBAX Eclipse Plus C18 3 × 50 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	0 min 20 % B
	3 min 100 % B
	3.3 min 100 % B
Flow rate	1.2 mL/min
Stop time	3.3 min
Post time	3 min
Injection volume	3 µL
Column temperature	40 °C
VWD	254 nm
Flow cell	10 mm
Peak width	> 0.003 min (80 Hz)

Column	Agilent ZORBAX Eclipse Plus C18 3 × 50 mm, 1.8 µm
Mobile phase	A = Water
	B = Acetonitrile
Gradient	0 min 20 % B
	1.5 min 100 % B
	1.7 min 100 % B
Flow rate	2.4 mL/min Pressure
Stop time	1.7 min
Post time	2 min
Injection volume	3 µL
Column temperature	50 °C
DAD	254 nm / 4 (360 nm / 100)
Flow cell	10 mm
Peak width	> 0.003 min (80 Hz)

Conclusion

The performance of the Agilent 1220 Gradient LC system fulfills all needs of modern analytical liquid chromatography. It is especially well suited for 3 mm and 4.6 mm id columns and can be used for conventional and for rapid resolution (RR) or ultra fast LC on columns packed with 1.8 µm particles.

Precision of retention times is typically </= 0.2% RSD. The precision for areas is typically < 0.25% for injection volumes >/= 5 µL. Carryover is typically < 0.05% with external needle cleaning. The VWD combines lowest noise (ASTM noise for the 10 mm path length cell was found to be ± 3.5 µAU) with a linear range up to 2 mAU.

www.agilent.com/chem/1220

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