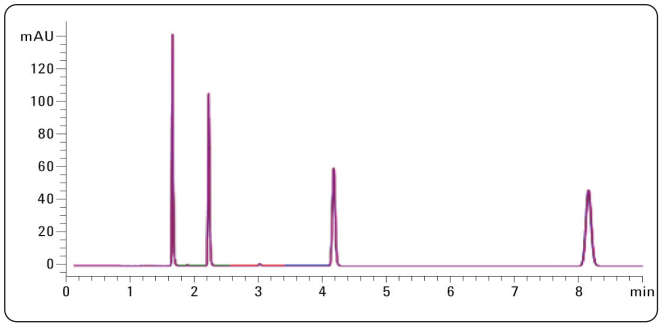
Performance characteristics of the 1260 Infinity Quaternary LC system

The new standard in HPLC

Technical Overview



Introduction

The Agilent 1260 Infinity LC system consists of modular units that operate with a maximum pressure of 600 bar. The complete Agilent 1260 Infinity LC portfolio comprises isocratic, binary and quaternary LC systems. The pump modules can be combined with a wide choice of detectors and autosampler modules. Specific solutions for purification or capillary columns are available.

The Agilent 1260 Infinity Quaternary LC system offers:

- Flow rate range up to 5 mL/min (required for ultrafast LC on standard bore columns with 3–4.6 mm id)
- Space for long HPLC columns (250–300 mm)
- Maximum pressure of 600 bar for Rapid Resolution LC or ultrafast LC on columns up to 150 mm length. Higher pressures allow support of either higher flow rates (more speed) or longer columns (more resolution)
- Maximum temperature of 80 °C (100 °C with an Agilent 1290 Infinity Thermostatted Column Compartment). Higher temperatures reduce pressures significantly thereby allowing higher flow rates for more speed on longer columns
- Diode Array Detector (DAD) with a new optical design, which uses a cartridge cell with optofluidic waveguide technology offering sensitivity with low dispersion.
- Standard bore RRLC and conventional applications run on the same system configuration.

Agilent Technologies

The following modules were tested:

The **Agilent 1260 Infinity Quaternary Pump** was tested for:

- Precision of retention time
- Performance of step gradients

The **Agilent 1260 Infinity Autosampler** was tested for:

- Precision of Areas
- Carryover
- Injection volume linearity

The **Agilent 1260 Infinity Diode Array Detector** was tested for:

- Noise and drift
- Limit of detection for Anthracene
- Linearity
- 60 mm path length cell vs. 10 mm path length cell

To ensure seamless method transfer from a 400 bar Agilent 1200 Series Quaternary system to a 600 bar Agilent 1260 Infinity Quaternary system, the tests checked:

- Influence on elution times
- Influence on signal-to-noise ratios
- Influence on resolution

Experimental

The Agilent Infinity Quaternary LC system that was tested consisted of:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity High performance Autosampler (G1367E)
- Agilent 1260 Infinity DAD (G4212B)
- Agilent 1260 Infinity Thermostatted Column Compartment (G1316A)

Pump performance - retention time precision

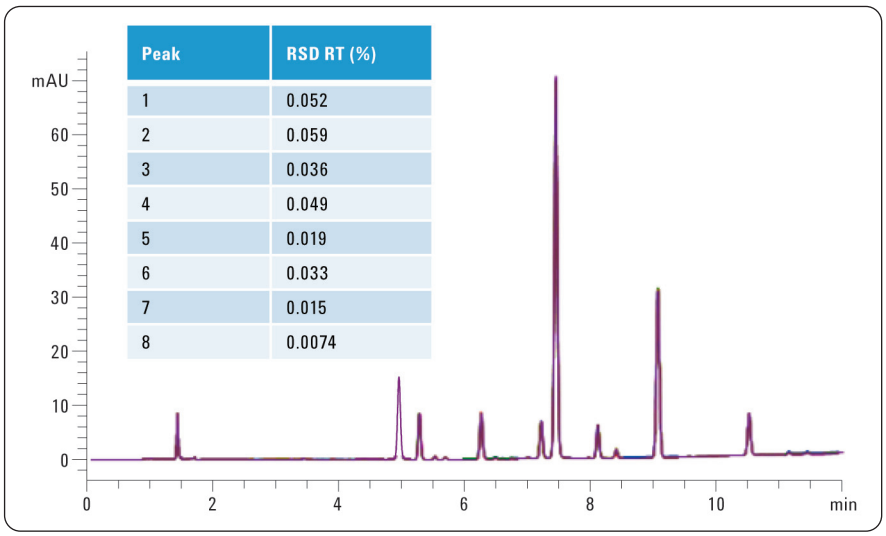
Optimum retention time precision depends mainly on:

- 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 pump performance; the other parameters influence precision of retention times. For example, if a solvent is changed, the column needs a minimum of 10 column volumes for proper equilibration. If gradients are applied, at least 5 column volumes are needed to equilibrate the column to the start conditions. If the column compartment temperature is changed from 30 °C to 60 °C, it takes about 20 min for the column to equilibrate to the new temperature. Proper degassing influences the precision positively.

Retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3 mm id columns. The relative standard deviation of retention times for conventional gradient runs was <0.06 % RSD (Figure 1).

For fast gradients with a run time of about 1 min, the relative standard deviation for retention times was < 0.21% RSD (Figure 2). Figure 3 shows conventional conditions with an RT precision of <0.07% RSD.



Peak	RSD RT (%)
1	0.052
2	0.059
3	0.036
4	0.049
5	0.019
6	0.033
7	0.015
8	0.0074

Figure 1
Precision of retention times for conventional gradient runs.

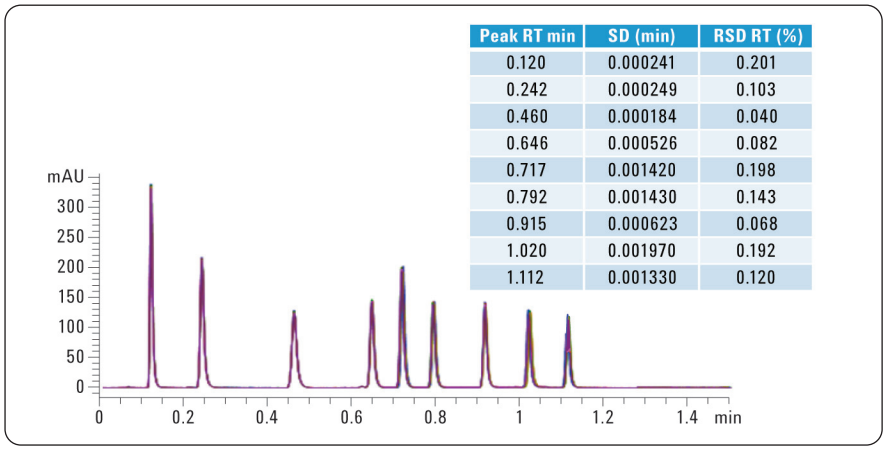
Chromatographic conditions

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, heptyl parabens, uracil)

Sample preparation:

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

Column:	Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 5 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 10 min 95% B
Flow rate:	1 mL/min
Stop time:	12 min
Post time:	5 min
Injection volume:	5 µL
Column temperature:	30 °C
DAD:	254/4 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

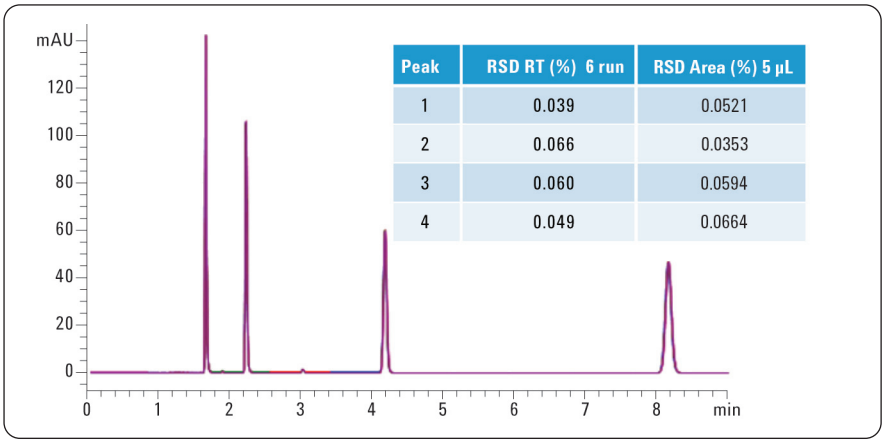


Peak RT min	SD (min)	RSD RT (%)
0.120	0.000241	0.201
0.242	0.000249	0.103
0.460	0.000184	0.040
0.646	0.000526	0.082
0.717	0.001420	0.198
0.792	0.001430	0.143
0.915	0.000623	0.068
1.020	0.001970	0.192
1.112	0.001330	0.120

Figure 2
Precision of retention times for fast gradient runs.

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 30% B 1 min 95% B
Flow rate:	3.5 mL/min
Stop time:	1.5 min
Post time:	1 min
Injection volume:	1 µL
Column temperature:	50 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.00625 min (40 Hz)



Peak	RSD RT (%) 6 run	RSD Area (%) 5 µL
1	0.039	0.0521
2	0.066	0.0353
3	0.060	0.0594
4	0.049	0.0664

Figure 3
RT precision for conventional isocratic runs.

Chromatographic conditions

Sample:	Isocratic sample (p/n 01080-68707)
Column:	Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 1.8 µm
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
DAD:	254/40 nm Ref 380/800 nm
Flow cell:	10 mm
Peak width:	<0.025 min, (10 Hz)

Performance of step gradient

Tracer experiments are frequently used to verify the solvent mixing ripple at different gradient mixtures to evaluate pump performance. The delay volume, 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. It is best to use non-volatile compounds for the Agilent 1260 Infinity pump step gradient performance testing.

The performance results are:

- Ripple on 10% step = 0.1%
- Ripple on 50% step = 0.11%
- Ripple on 90% step = 0.11%
- Precision of step height for 50% step = 0.1% RSD for 3 runs
- System delay volume: 950 µL at 322 bar

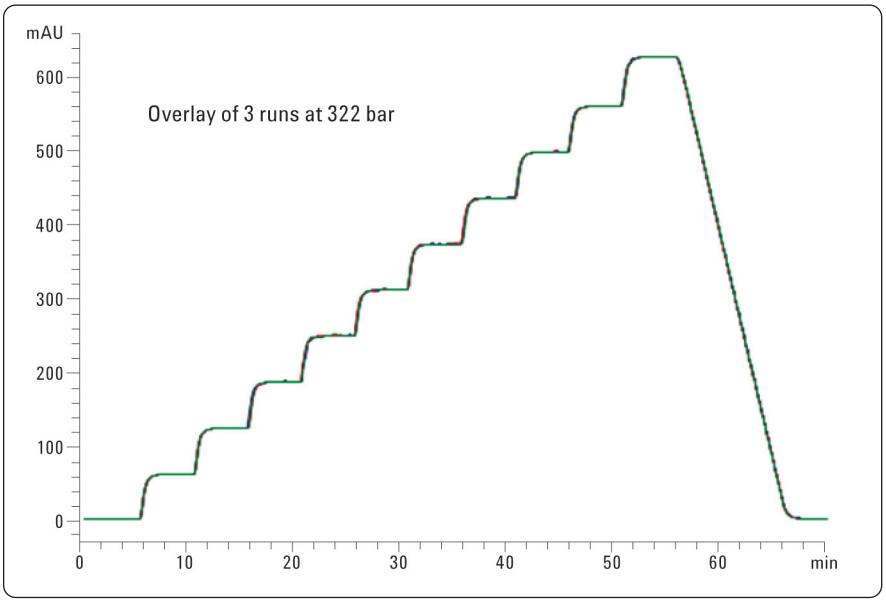


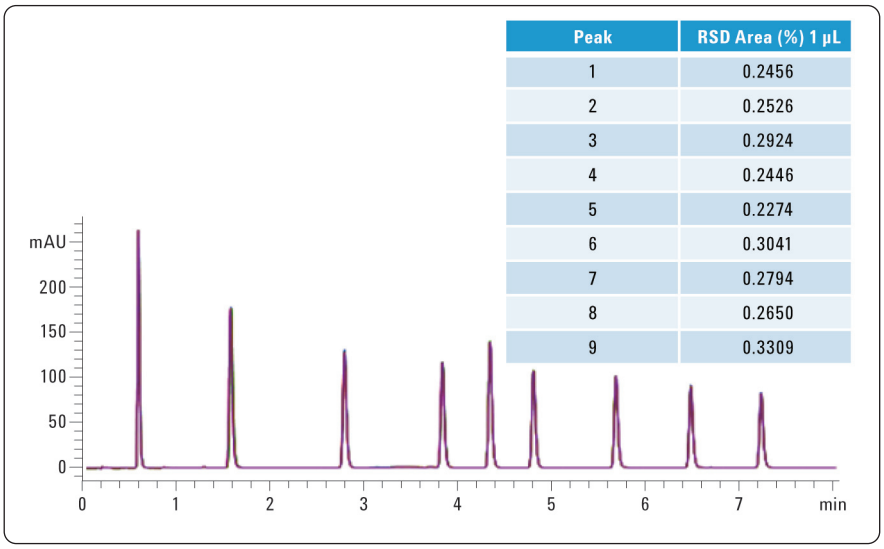
Figure 4
Overlay of three consecutive step gradient runs.

Chromatographic conditions

Column:	Restriction Capillary
Mobile phase:	A = Water + 20% Isopropanol B = Water + 20% Isopropanol + 10 mg/L caffeine
Step Gradient:	From 0 to100% B in 10 % steps
Flow rate:	1 mL/min
Stop time:	70 min
Post time:	5 min
Column temperature:	36 °C
DAD:	273/4 nm Ref 380/100 nm, slit 4
Flow cell:	10 mm
Peak width:	<0.0125 min, (20 Hz)

Injector performance - area precision

Precise injection is mandatory for good quantitative results in liquid chromatography. The Agilent 1260 Infinity High Performance Autosampler can inject precisely over an injection range of 0.5 to 100 µL. Figure 5 shows an example chromatogram for an injection volume of 1 µl. The relative standard deviation is <0.34%.



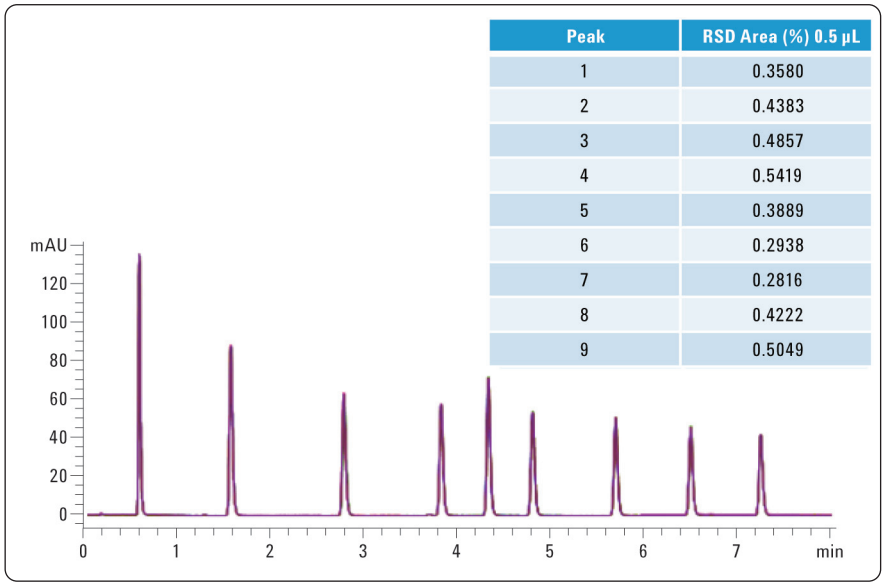
Peak	RSD Area (%) 1 µL
1	0.2456
2	0.2526
3	0.2924
4	0.2446
5	0.2274
6	0.3041
7	0.2794
8	0.2650
9	0.3309

Figure 5
Area precision for conventional run for 1 µL.

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

Figure 6 shows an example chromatogram for an injection volume of 0.5 µL. The relative standard deviation is <0.55%.



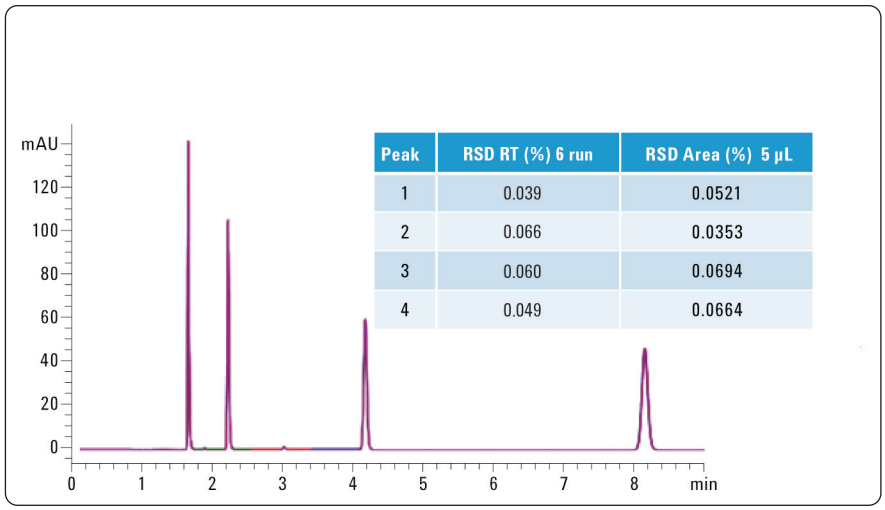
Peak	RSD Area (%) 0.5 µL
1	0.3580
2	0.4383
3	0.4857
4	0.5419
5	0.3889
6	0.2938
7	0.2816
8	0.4222
9	0.5049

Figure 6
Precision of areas for 0.5 µL injection.

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	0.5 µL
Column temperature:	30 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

Figure 7 shows an example chromatogram for an injection volume of 5 µL. The relative standard deviation is <0.07%.



Peak	RSD RT (%) 6 run	RSD Area (%) 5 µL
1	0.039	0.0521
2	0.066	0.0353
3	0.060	0.0694
4	0.049	0.0664

Figure 7
Area precision for isocratic runs with 5 µL injection volume.

Chromatographic conditions

Sample:	Isocratic sample (p/n 01080-68707)
Column:	Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 1.8 µm
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
DAD:	254/40 nm Ref 380/80 nm
Flow cell:	10 mm
Peak width:	<0.025 min, (10 Hz)

The injector settings are very important for optimum precision of areas. For example, if the highest precision is needed, the draw speed of the injector should be set to lower values, especially if large volume or viscous samples are injected. It is also important to avoid solvent evaporation out of the sample vials. Use cooled autosamplers to avoid evaporation and decomposition problems in the sample vials. If a sample may be reused, the vial should be freshly capped for storage.

Carryover

Carryover was tested using the Agilent 1260 Infinity High Performance Autosampler. The injection draw speed was set to 20 µL/min and an exterior needle wash for 10 sec was used. The carryover (Figure 8) was found to be <0.004% (40 ppm) for the conditions used. After a 1000-ng sample injection, unadulterated solvent was injected.

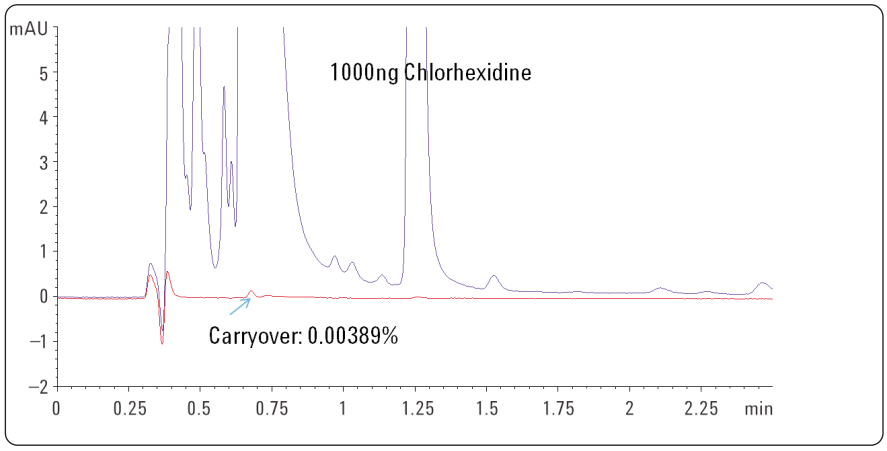


Figure 8
Carryover = 0.00389% after injection of 1000 ng Chlorhexidine.

Chromatographic conditions

Sample:	Chlorhexidine 1000 ng/µL dissolved in 0.1% Water TFA
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	40% B
Flow rate:	0.6 mL/min
Stop time:	2.5 min
Injection volume:	1 µL, draw speed 20 µL/min, 10 sec wash for exterior of needle
Column temperature:	50 °C
DAD:	254/4 nm Ref 360/100 nm
Flow cell:	10 mm
Peak width:	<0.0125 min (20 Hz)

Recommendations for carryover and cleaning procedures

Flush port wash solvent must always be installed and used. The solvent chosen should be able to dissolve the sample compounds. If the wash bottle is empty, the flush port is not primed, or the flush port is not cleaned correctly, the injector must be cleaned by back-flushing the seat, seat capillary and valve groove. It is also highly recommended to use freshly installed capillary connections whenever the column is changed. This is important because any unswept volume will give additional carryover.

To backflush the seat and seat capillary, lift the needle and disconnect the capillary coming from the pump at the injection valve using ChemStation. Connect the capillary to port 4 of the injection valve. Start pumping with 5 mL and flush for about 2 min. The instrument is ready to restore method conditions and begin analysis.

Injection volume linearity

Injection volume linearity was tested using Primidone standards. All injection volumes contained 781.26 ng of Primidone. This means the injection volume varied but the injected amount was always the same (Figure 9). The peak heights and areas should be the same for all injection volumes. The experiments showed that all areas were within 1.35% RSD over the complete injection volume range of 0.8 to 100 µL. Each injection volume was injected three times and the 24 runs were evaluated for area precision. The sample wavelength was set to 254 nm, which is in the low absorbance range of the spectrum. Evaluation of data obtained at 220 nm, which is the higher absorbance range, showed nearly the same results.

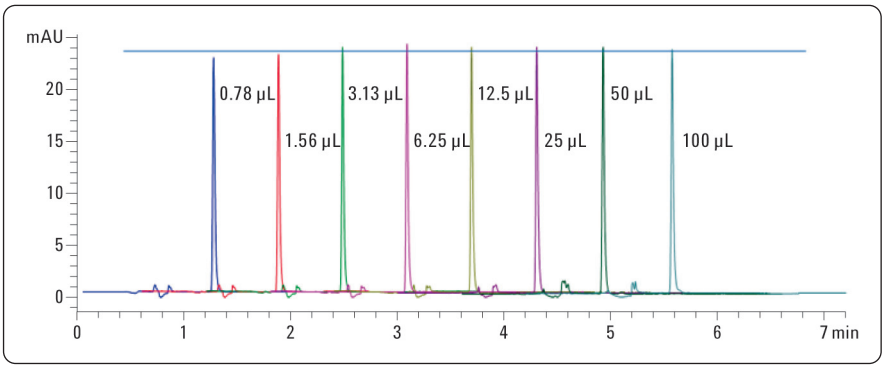


Figure 9
Injection volume linearity from 0.8 up to 100 µL; injected amount was always the same.

Chromatographic conditions

Sample:	Primidone 250 ng/25 mL 7 times 1:2 diluted
Column:	Agilent ZORBAX Eclipse Plus C18, 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:	50 °C
DAD:	254/4 nm Ref 380/80 nm
Flow cell:	10 mm
Peak width:	<0.0125 min (20 Hz)

It is very important to prepare an accurate dilution series to obtain good linearity. One way is to dilute large volumes, such as 1 liter. Ensure special care is used when small volumes are diluted. The pipettes should be calibrated and the same pipette always used for the complete dilution series. Otherwise there is a risk that dilution error is measured rather than linearity.

Performance of the Agilent 1260 Infinity DAD

Baseline noise ASTM and drift for the 10 mm and 60 mm path length cell

The ASTM noise and drift were evaluated using a restriction capillary column and water as the mobile phase. The detector was set to a 4-sec response time. The resulting ASTM noise for the 10 mm path length cell was found to be Noise = ±1.76 µAU and the drift was Drift = 0.1623 mAU/h. The resulting ASTM noise for the 60 mm path length cell was found to be Noise = ± 2.411 µAU/ and the drift was Drift = –0.4394 mAU/h. Figure 10 shows example chromatograms for the noise and drift behavior of both cells.

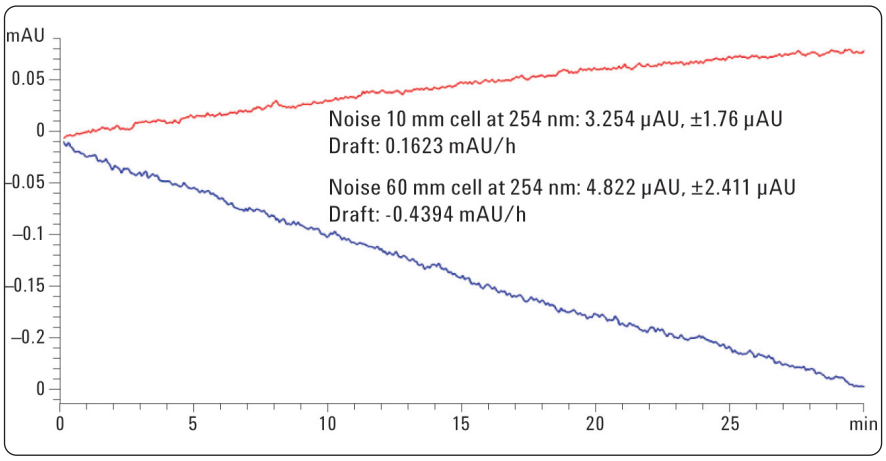


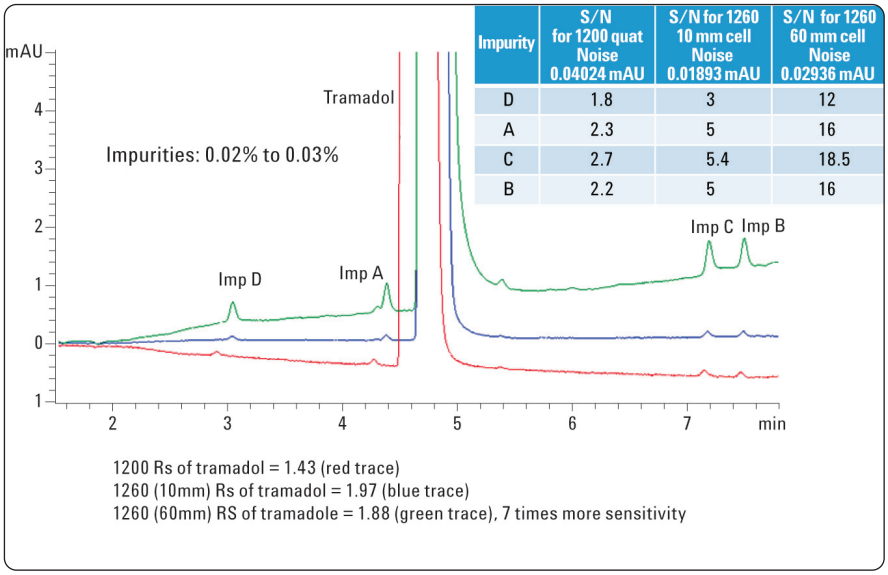
Figure 10
Noise and drift for the 10 mm and 60 mm path length cell.

Chromatographic conditions

Column:	Restriction Capillary with 162 bar back pressure
Mobile phase:	A = Water
Flow rate:	1 mL/min
Stop time:	30 min
Column temperature:	36 °C
DAD:	254/4 nm Ref 380/100 nm, slit 4
Flow cell:	10 mm and 60 mm
Peak width:	<0.02 min, 4 sec response time (1.25 Hz)

Higher sensitivity with the Agilent 1260 Infinity DAD using the 60 mm path length cell

Figure 11 illustrates an increase in sensitivity if the 60 mm path length cell is used. Tramadole was used as a sample. Tramadol contains 4 UV active impurities in the range of 0.02% to 0.03%. Figure 11 shows an overlay of the chromatograms obtained on an Agilent 1200 Infinity Series Quaternary system, an Agilent 1260 Infinity Quaternary LC system with 10 mm path length cell, and an Agilent 1260 Infinity Quaternary LC system with a 60 mm path length cell. The resolution for tramadol on the Agilent 1200 Infinity Series Quaternary LC system (red trace) was found to be Rs= 1.43. On the Agilent 1260 Infinity Quaternary LC system with 10 mm cell (blue trace) the resolution was Rs = 1.97 and for the 60 mm cell (green trace) the resolution was Rs = 1.88. The 60 mm cell on the Agilent 1260 Infinity Quaternary LC system increased the signal-to-noise ratio to seven times that of the Agilent 1200 Series Quaternary LC system.



Impurity	S/N for 1200 quat Noise 0.04024 mAU	S/N for 1260 10 mm cell Noise 0.01893 mAU	S/N for 1260 60 mm cell Noise 0.02936 mAU
D	1.8	3	12
A	2.3	5	16
C	2.7	5.4	18.5
B	2.2	5	16

Figure 11
Seven times increase in sensitivity for the Agilent 1260 Infinity Quaternary LC system than the Agilent 1200 Series Quaternary LC system.

Chromatographic conditions

Sample:	Tramadol 4.2 mg/1 mL Water/ACN = 50/50
Column:	Agilent ZORBAX Eclipse Plus C18, 150 mm × 3.0 mm 5 µm, 400 bar
Mobile phase:	A = Water +0.05% TFA B = Acetonitrile +0.045% TFA
Gradient:	At 0 min, 15% B 8 min, 45% B
Flow rate:	0.8 mL/min
Stop time:	8.5 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	270/4 nm Ref 380/100 nm, slit 4
Flow cell:	10 mm and 60 mm path length cell
Peak width:	<0.0125 min (20 Hz)

Linearity for different caffeine concentrations

The linearity was tested using caffeine standards from 1.5 ng to 2000 ng 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 over an absorbance range of 2 up to 2290 mAU (Figure 12).

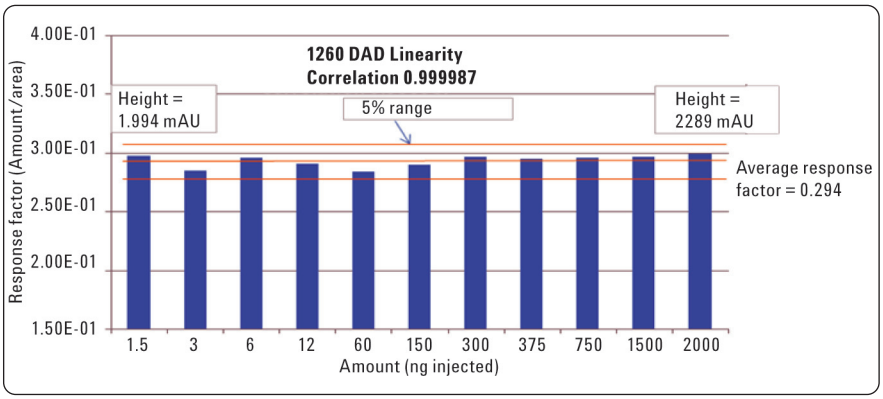


Figure 12
Linearity of the Agilent 1260 Infinity DAD

Chromatographic conditions

Column:	Agilent Poroshell 120 EC C18, 3.0 mm × 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:	3 µL, 4 µL (2000 ng)
Column temperature:	30 °C
DAD:	273/10 nm Ref 380/80 nm
Flow cell:	10 mm
Peak width:	<0.0125 min (20 Hz)

Method transfer

Seamless method transfer from an Agilent 1200 Series Quaternary LC system to an Agilent 1260 Infinity Quaternary LC system is important for backwards compatibility. To avoid significant differences in the resulting chromatograms, the delay volume of both systems should be similar and the formation of gradients should be comparable. Three experiments were performed to demonstrate that a method developed on the Agilent 1200 Series Quaternary system can be transferred to an Agilent 1260 Infinity Quaternary system without significant differences in the chromatogram. In this analysis, we prove that there is no significant difference in the appearance of the chromatograms for tramadol analysis (Figure 11). The sensitivity performance is typically better, especially if the 60 mm path length detector cell is used.

In another example, a 150 mm × 4.6 mm, 5 µm column was used on both instruments. A 10-min gradient was applied and a flow rate of 1 mL/min was used. The resulting pressure was 70 bar. Figure 13 shows an overlay of the obtained chromatograms. No significant differences could be observed.

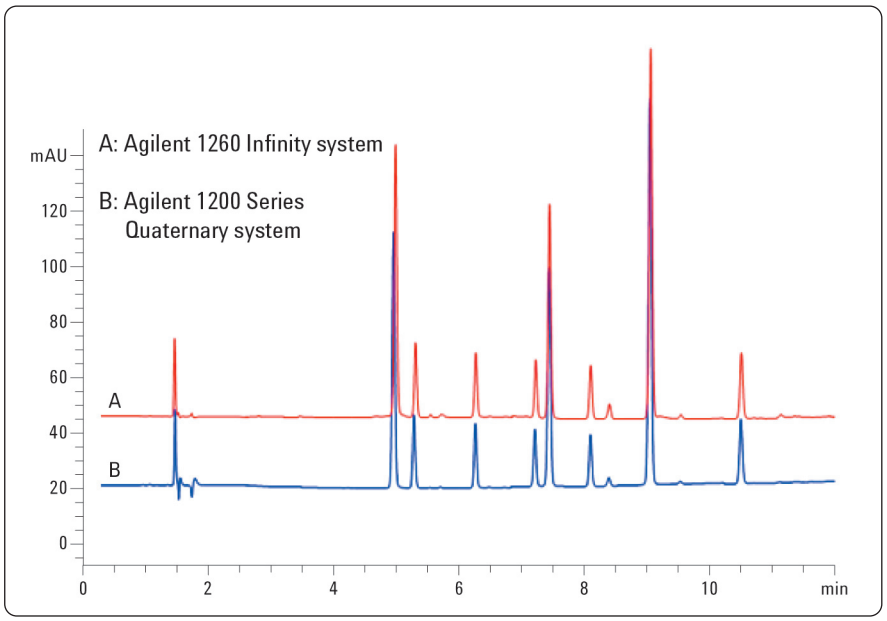


Figure 13
Overlay of chromatograms obtained on the Agilent 1260 Infinity Quaternary and the Agilent 1200 Series Quaternary system at approximately 70 bar back pressure.

Instruments

Agilent 1200 Series LC system

- Pump G1311A
- Degasser G1322A
- Autosampler G1329A
- Thermostatted Column Compartment G1316A
- Diode Array Detector G1315D

Agilent 1260 Infinity LC system

- Pump G1311B
- Autosampler G1329B
- Thermostatted Column Compartment G1316B
- Diode Array Detector G4212A

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, heptyl parabens, uracil)

Sample preparation

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

Column:	Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 5 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 10 min 95% B
Flow rate:	1 mL/min
Stop time:	12 min
Post time:	5 min
Injection volume:	5 µL
Column temperature:	30 °C
DAD:	254/4 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

If higher pressures were applied the retention time changes did not exceed 1.5%. The applied pressure was approximately 300 bar (Figure 14).

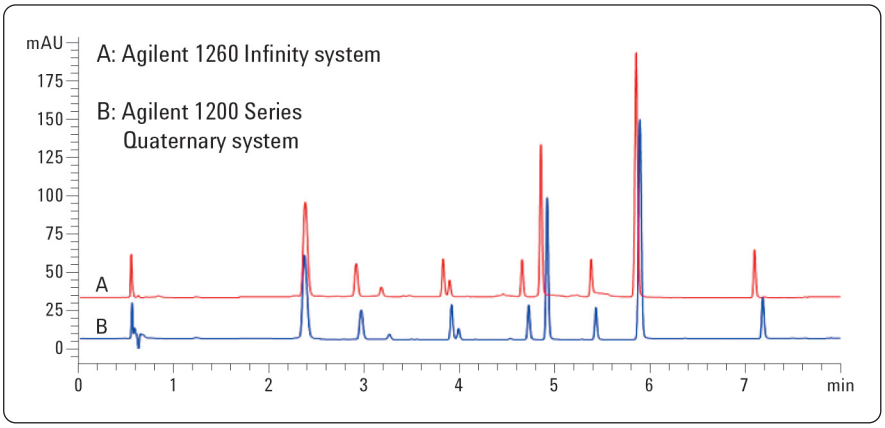


Figure 14
Overlay of chromatograms obtained on the Agilent 1260 Infinity Quaternary system and the Agilent 1200 Infinity Series Quaternary system at approximately 300 bar back pressure.

Instruments

Agilent 1200 Series LC system

- Pump G1311A
- Degasser G1322A
- Autosampler G1329A
- Thermostatted Column Compartment G1316A
- Diode Array Detector G1315D

Agilent 1260 Infinity LC system

- Pump G1311B
- Autosampler G1329B
- Thermostatted Column Compartment G1316B
- Diode Array Detector G4212A

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, heptyl parabens, uracil)

Sample preparation

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

Column:	Agilent ZORBAX SB C18, 3 mm × 100 mm, 1.8 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 20% B 7 min 95% B
Flow rate:	0.7 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	5 µL
Column temperature:	50 °C
DAD:	254/4 nm Ref 400/100 nm
Flow cell:	10 mm
Peak width:	<0.025 min (10 Hz)

Conclusion

The performance of the Agilent 1260 Infinity Quaternary LC system meets the requirements of modern analytical liquid chromatography. It is well suited for 3 mm and 4.6 mm id columns, and can be used for conventional and rapid resolution (RR) or ultrafast LC on columns packed with 1.8 µm particles.

Precision of retention times for conventional LC is typically $\leq$ 0.07% RSD. The precision for areas is typically < 0.25% for injection volumes $\geq$ 5 µL. Carryover is typically <0.004%. The DAD achieves the lowest noise. ASTM noise for the 10 mm path length cell was found to be Noise = ±1.76 µAU, with a wide linear range up to 2300 mAU. Method transfer from an Agilent 1200 Infinity Series system to an Agilent 1260 Infinity Quaternary LC system will typically result in chromatograms with no significant differences.

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