Performance characteristics of the Agilent 1290 Infinity Quaternary Pump

Technical Overview

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Abstract

This Technical Overview presents Proof of Performance for the new Agilent 1290 Infinity Quaternary Pump. The 1290 Infinity Quaternary Pump combines the performance of a binary high-pressure mixing UHPLC pump with the flexibility of a low-pressure quaternary pump for conventional or fast runs on 3- and 4.6-mm id columns in addition to applications that need 2.1-mm id columns. Precision of retention times as well as precision and accuracy of step gradients were evaluated and compared to results obtained on the Agilent 1290 Infinity Binary and 1260 Infinity Quaternary Pumps.

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Introduction

The Agilent 1290 Infinity Quaternary Pump provides excellent performance through innovative design elements such as:

- Integrated, high efficiency degassing unit with low internal volume facilitates fast change-over of solvents when purging and priming the pump

- Highest solvent mixing efficiency before solvent reaches the pump head through the new Agilent Inlet Weaver, based on established multilayer mixing technology

- Active damping with independently controllable high resolution pump drives and firmware-embedded tuning algorithms significantly reduce ripples and associated UV noise

- Optional 380-µL Agilent Jet Weaver for extra mixing volume

- Inline filter in front of the injector protects the column from contamination

- Multipurpose valve enables automatic software-embedded functionality :

  - Switching the mixer in and out of flow path

  - Backflushing the inline filter

  - Automatic purging

Figure 1 shows the design of the 1290 Infinity Quaternary Pump.

This Technical Overview evaluated the following important pump parameters:

- The system delay volume and the mixing noise were determined based on step gradient tracer experiments.

- The composition accuracy and precision were determined based on step gradient tracer experiments. The precision of retention times was evaluated under different chromatographic conditions.

Results obtained on the Agilent 1290 Infinity Binary Quaternary Pump and the 1260 Infinity Quaternary Pump were compared.

Experimental

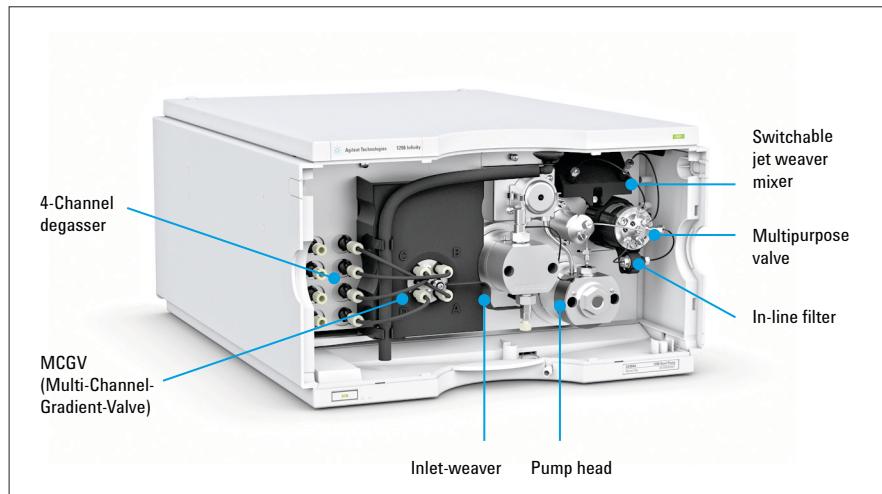The instruments used are listed in Table 1.

Figure 1
Agilent 1290 Infinity Quaternary Pump.

Module	Agilent 1290 Infinity LC System Product number	Agilent 1260 Infinity LC Product number
Binary pump	G4220A	
Quaternary pump	G4204A	G1311B
Autosampler	G4226A	G1329B
Autosampler thermostat	G1330B	G1330B
Thermostatted column compartment	G1316C	G1316C
Diode array detector	G4212A	G4212B

Table 1
Instruments used for the performance experiments.

Chromatographic conditions for step gradients

Column substitute:	Restriction capillaries providing a backpressure of approximately 170 bar
Mobile phases:	A = water +20% isopropanol, B = water +20% isopropanol +10 mg/L caffeine
Flow rate:	1 mL/min
Restrictor temperature:	36 °C
Detection:	Signal 273 nm/4 nm bandwidth, Ref 380/100 nm, Slit 4 nm, 20 Hz
Step gradient 1:	0% B to 100% B in 10% steps, each step held for 5 minutes
Step gradient 2:	0% B to 10% B in 1% steps, each step held for 5 minutes

Chromatographic conditions for conventional gradient runs on a 4.6-mm id column

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 × 150 mm, 5 µm (p/n 7995218-595)
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	0 minutes 20% B, at 10 minutes 95% B
Flow rate:	1 mL/min , maximum backpressure of 125 bar
Stop time:	12 minutes
Post time:	5 minutes
Injection volume:	5 µL, exterior needle wash 3 seconds
Column temperature:	30 °C
DAD:	254/4 nm, Ref 400/100 nm, Flow cell: 10 mm, Peak width: <0.025 minutes (10 Hz)

Chromatographic conditions for conventional isocratic runs on a 4.6-mm id column

Sample:	Isocratic sample (p/n 01080-68707)
Column:	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 1.8 µm (p/n 829975-902)
Mobile phase:	A = Water, B = Acetonitrile
Isocratic:	30/70 = A/B
Flow rate:	1.2 mL/min
Stop time:	8 minutes
Injection volume:	1 µL, exterior needle wash 3 seconds
Column temperature:	40 °C
DAD:	254/40 nm, Ref 380/80 nm, Peak width: <0.025 minutes, (10 Hz)

Chromatographic conditions for fast runs on a 3-mm id column

Sample:	RRLC Checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm (p/n 699975-302)
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	0 minutes 30% B, 1 minute 95% B
Flow rate:	3 mL/min, 550 bar max pressure
Stop time:	1.5 minutes
Post time:	1 minute
Injection volume:	1 µL, exterior needle wash 3 seconds
Column temperature:	60 °C
DAD:	245/10 nm, Ref 400/100 nm, Flow cell: 10 mm, Peak width: <0.00625 minutes (40 Hz)

Chromatographic conditions for conventional runs on a 2.1-mm id column

Column:	Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm (p/n 959758-902)
Sample:	

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.

Mobile phase:	A = Water, B = Acetonitrile
Gradient:	At 0 minutes 10% ACN, at 10 minutes 90% ACN
Flow rate:	0.3 mL/min at 830 bar max pressure
Oven temperature:	50 °C
DAD:	254/10 nm, REF 360/100 nm, 20 Hz
Injection volume:	1 µL, exterior needle wash 3 seconds

Chromatographic conditions for fast runs on a 2.1-mm id column

Column:	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Sample:	

Set of 9 compounds, 100 ng/µL 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

Mobile phase:	A = Water, B = Acetonitrile
Gradient:	At 0 minutes 10% ACN, at 1 minute 95% ACN
Flow rate:	1 mL/min
Oven temperature:	45 °C
DAD:	245/10 nm, REF 360/80 nm, 80 Hz
Injection volume:	0.5 µL exterior needle wash 3 seconds

Software

Agilent OpenLAB CDS ChemStation Edition C.01.04

Results and discussion

The system delay volume, the mixing noise, the composition accuracy and the composition precision were measured using step gradients. For these experiments, the 1290 Infinity Quaternary Pump was used without optional mixer.

Experiment 1

Two step gradient experiments were performed:

- From 0 to 100% tracer in 10% steps, each step held for 5 minutes

- From 0 to 10% tracer in 1% steps, each step held for 5 minutes

The same step gradient conditions were applied to the 1290 Infinity Quaternary and Binary Pump and the 1260 Infinity Quaternary Pump. Figure 2 shows the step gradient from 0% tracer up to 100% tracer in 10% steps for the three instruments.

Table 2 summarizes the results for the three step gradients. The 1290 Infinity Quaternary Pump provided comparable results with the 1290 Infinity Binary Pump except for the delay volume and the composition accuracy. The 1260 Infinity Quaternary Pump also showed excellent results, but the system delay was significantly larger compared to the other two LC systems.
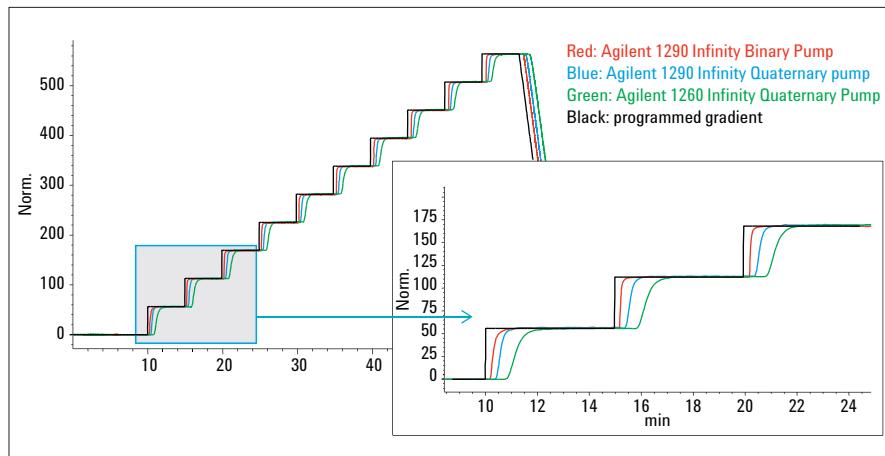

Red: Agilent 1290 Infinity Binary Pump
Blue: Agilent 1290 Infinity Quaternary pump
Green: Agilent 1260 Infinity Quaternary Pump
Black: programmed gradient

Figure 2
Step gradient from 0 to 100% tracer in 10% steps on an Agilent 1290 Infinity Binary and 1290 Infinity Quaternary Pump and on an Agilent 1260 Infinity Quaternary Pump.

	1260 Infinity Quaternary Pump	1290 Infinity Quaternary Pump	1290 Infinity Binary Pump
Ripple at 10% step (related to 100% step)	0.08%	0.1%	0.12%
Ripple at 50% step	0.08%	0.1%	0.2%
Ripple at 90% step	0.08%	0.06%	0.05%
Precision of 10% step (for three cons. runs)	0.18% RSD	0.1% RSD	0.14% RSD
Precision of 50% step (for three cons. runs)	0.06%RSD	0.02%	0.002% RSD
System delay volume	approx. 900 µL	approx. 420 µL	approx. 170 µL
Composition accuray (specification)	NA	±0.4%	±0.35%

Table 2
Step gradient results.

The wide gradient range of the 1290 Infinity Quaternary Pump from 0 to 100% provided an excellent step gradient profile even from 0 to 10% tracer in 1% steps. Steps 1, 2, and 3 were well defined, see Figure 3 for the 1290 Infinity Binary Pump. The 1260 Infinity Quaternary Pump provided optimum results from the 3% step onwards. This is reflected in the specified range from 5 to 95% organic for the 1260 Infinity Quaternary Pump.

The step gradient profiles proved that the 1290 Infinity Quaternary Pump showed a similar profile as the 1290 Infinity Binary Pump even for the demanding range from 0 to 3% tracer. The 1260 Infinity Quaternary Pump profile was different especially at the steps from 0 to 3%.

Precision of retention times was evaluated applying different chromatographic conditions on all three pumps using five additional experiments:

Experiment 2:
Conventional gradient runs on 4.6 × 150-mm column applying 1 mL/min flow rate

Experiment 3:
Conventional isocratic runs on 4.6 × 150-mm id column applying 1.2 mL/min flow rate

Experiment 4:
Fast gradient runs on 3 × 50-mm column applying 3 mL/min flow rate

Experiment 5:
Conventional gradient runs on 2.1 × 100-mm column applying 0.3 mL/min flow rate

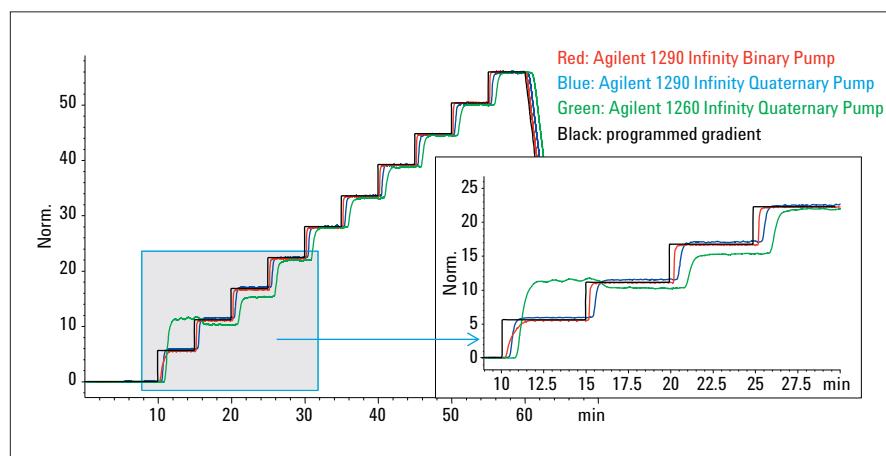Experiment 6:
Fast gradient runs on 2.1 × 50-mm column applying 1.2 mL/min flow rate

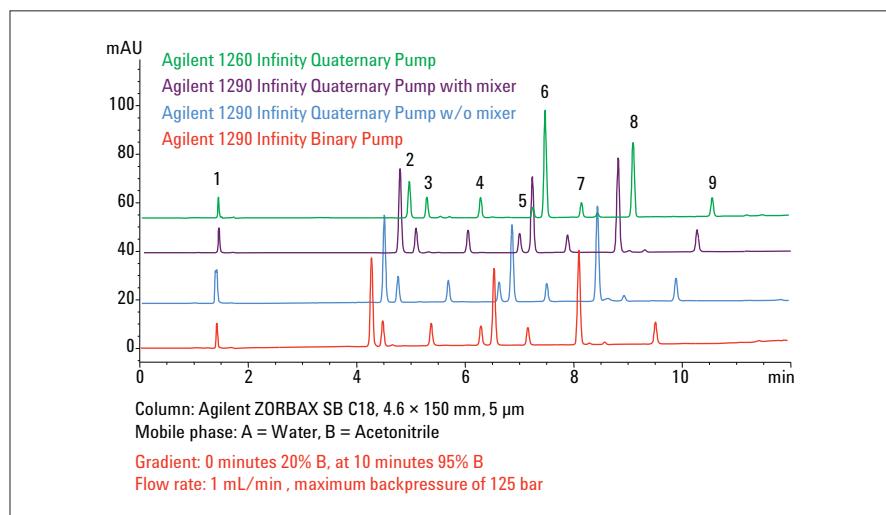The precision data are based for all experiments on six consecutive runs.

Experiment 2

Conventional gradient runs were done on a ZORBAX SB-C18 4.6 x 150 mm, 5-µm column with 1 mL/min flow rate. Figure 4 shows an overlay of the chromatograms obtained using the 1290 Infinity Quaternary Pump with and without the optional mixer, 1290 Infinity Binary Pump and the 1260 Infinity Quaternary pump. The lowest delay volume was achieved by the 1290 binary pump, as expected, followed by the 1290 Infinity Quaternary Pump without the optional mixer.

Figure 3
Step gradient from 0 to 10% tracer in 1% steps on an Agilent 1290 Infinity Binary and Quaternary Pump and on an Agilent 1260 Infinity Quaternary Pump.
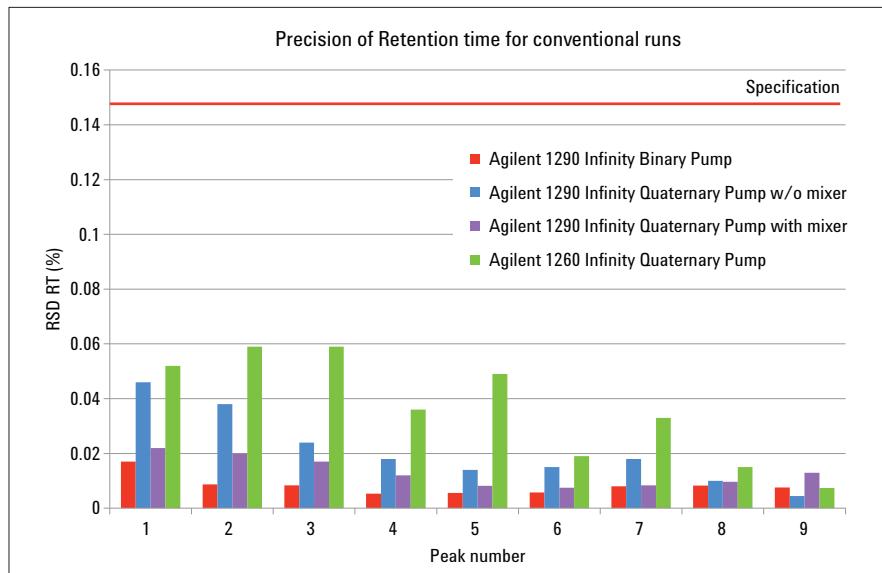

Figure 4
Overlay of the four chromatograms applying the conventional gradient, 1. uracil, 2. phenol, 3. methyl paraben, 4. ethylparaben, 5. propylparaben, 6. n,n-diethyl-m-toluamide, 7. butylparaben, 8. toluene, 9. heptylparaben.

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Regarding retention time precision, the 1290 Infinity Quaternary Pump with mixer and the 1290 Infinity Binary Pump showed comparable performance, see Figure 5. The 1290 Infinity Quaternary Pump without mixer also provided excellent precision data. The 1260 Infinity Quaternary Pump showed very good results, which were far below the 1290 Infinity Quaternary Pump specification.
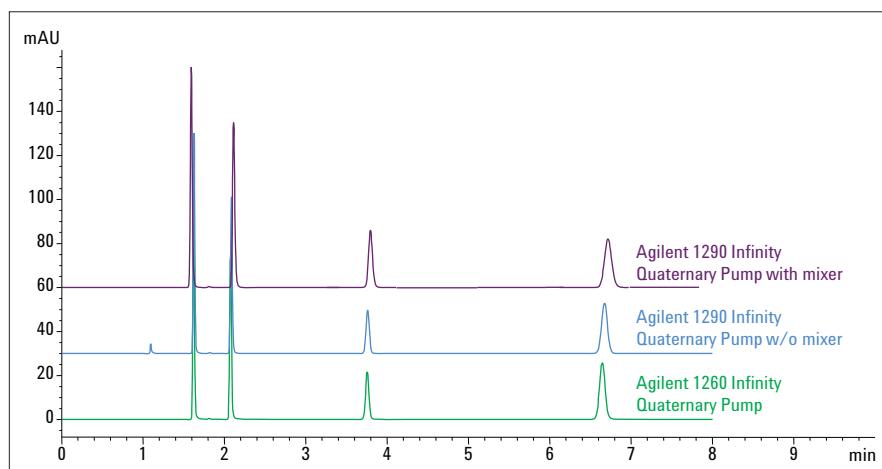
The relative standard deviation range of the retention times for the 1290 Infinity Quaternary Pump with optional mixer ranged from 0.022 down to 0.0075% RSD and without mixer from 0.046 to 0.00444% RSD. The range for the 1290 Infinity Binary Pump was 0.0053 to 0.017% RSD, and the range for the 1260 Infinity Quaternary Pump was 0.0074 to 0.059% RSD.

Experiment 3

Conventional isocratic runs were done applying 1.2 mL/min on an Agilent ZORBAX SB-C18, 4.6 × 150 mm, 1.8 µm column. These conventional isocratic conditions were applied on the 1260 Infinity Quaternary Pump, and on the 1290 Infinity Quaternary Pump without and with the optional mixer, see Figure 6. As expected, after applying isocratic conditions, the retentions did not differ for the three instrument configurations.

Figure 5
Precision of retention times for the Agilent 1290 Infinity Binary and Quaternary Pump (with and w/o optional mixer) and for the Agilent 1260 Infinity Quaternary Pump.
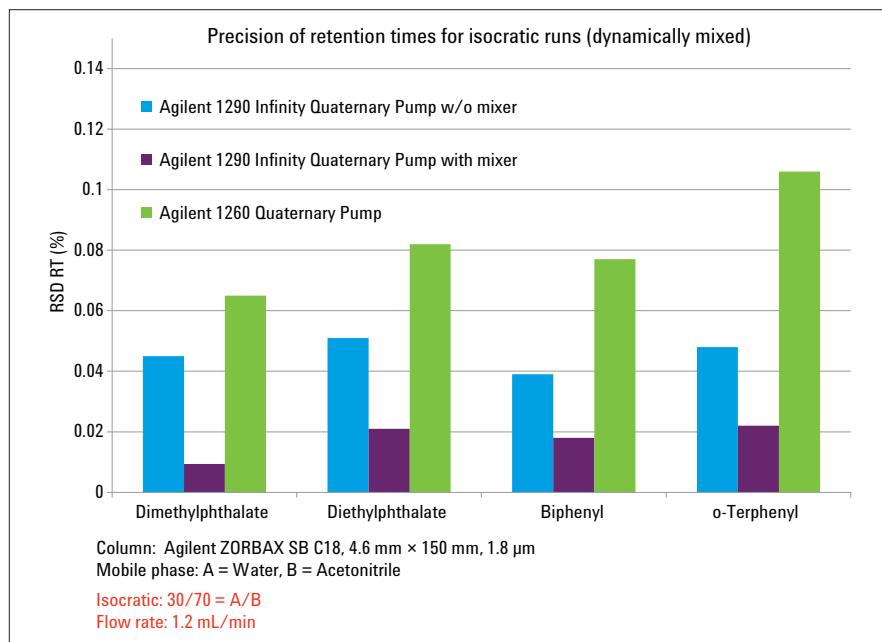

Figure 6
Overlay of chromatograms using conventional isocratic conditions, 1. dimethylphthalate, 2. diethylphthalate, 3. biphenyl, 4. o-terphenyl.

The maximum standard deviation of retention times for the 1290 Infinity Quaternary Pump was 0.01% RSD with a mixer and 0.051% RSD without a mixer for dynamically mixed mobile phases, see Figure 7. The 1260 Infinity Quaternary Pump showed a maximum relative standard deviation for the retention times of 0.106% RSD.
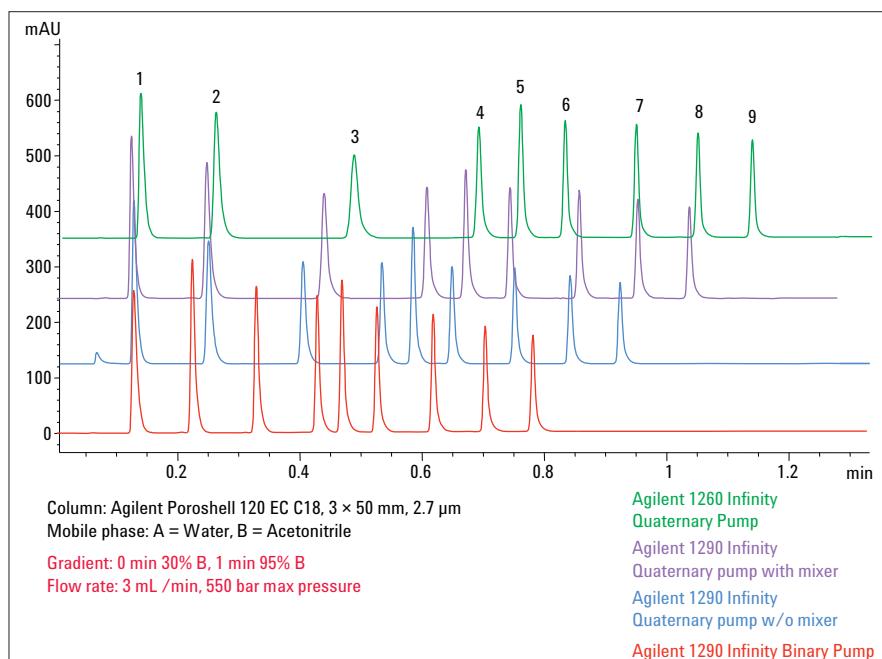
Experiment 4

Fast runs below 1.2 minutes run time were done on an Agilent Poroshell 120 EC C18, 3 × 50 mm, 2.7 µm column applying a flow rate of 3 mL/min. All four instrument configurations were tested with these demanding chromatographic conditions. The configuration with the lowest delay volume, the 1290 Infinity Binary LC, provided the shortest run times, see Figure 8.

Whether the first peaks eluted isocratic or were affected by the gradient was determined by the delay volume of the used LC instrument.

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

Figure 7
Precision of retention times for isocratic conditions comparing an Agilent 1290 Infinity Quaternary and an Agilent 1260 Infinity Quaternary pump.

Column: Agilent Poroshell 120 EC C18, 3 × 50 mm, 2.7 µm
Mobile phase: A = Water, B = Acetonitrile
Gradient: 0 min 30% B, 1 min 95% B
Flow rate: 3 mL /min, 550 bar max pressure

Agilent 1260 Infinity Quaternary Pump
Agilent 1290 Infinity Quaternary pump with mixer
Agilent 1290 Infinity Quaternary pump w/o mixer
Agilent 1290 Infinity Binary Pump

Figure 8
Overlay of chromatograms obtained using high flow rate, 1. Acetanilide, 2. Acetophenone, 3. Propiophenone, 4. Butyrophenone, 5. Benzophenone, 6. Valerophenone, 7. Hexanophenone, 8. Heptanophenone, 9. Octanophenone.

The precision for all instrument configurations was excellent for these demanding chromatographic conditions. Nevertheless, the 1260 Infinity Quaternary LC configuration did not show the same extremely good overall precision of retention times as the 1290 Infinity Binary and Quaternary LC configurations. The 1290 Infinity with mixer and the binary pump showed comparable results, see Figure 9.

The relative standard deviation range of the retention times for the 1290 Infinity Quaternary Pump was 0.207 to 0.0057% RSD with mixer and 0.132 to 0.022% RSD without mixer. The range for the 1290 Infinity Binary Pump was 0.214 to 0.0076% RSD and the range for the 1260 Infinity Quaternary Pump was 0.209 to 0.028% RSD.

The first peak had a retention time as short as approximately 0.130 minutes and the standard deviation was as low as 0.0103 seconds, which is far below the specification, see Figure 10. Considering these extremely low standard deviation values, the relative standard deviation results were emphasized impressively.
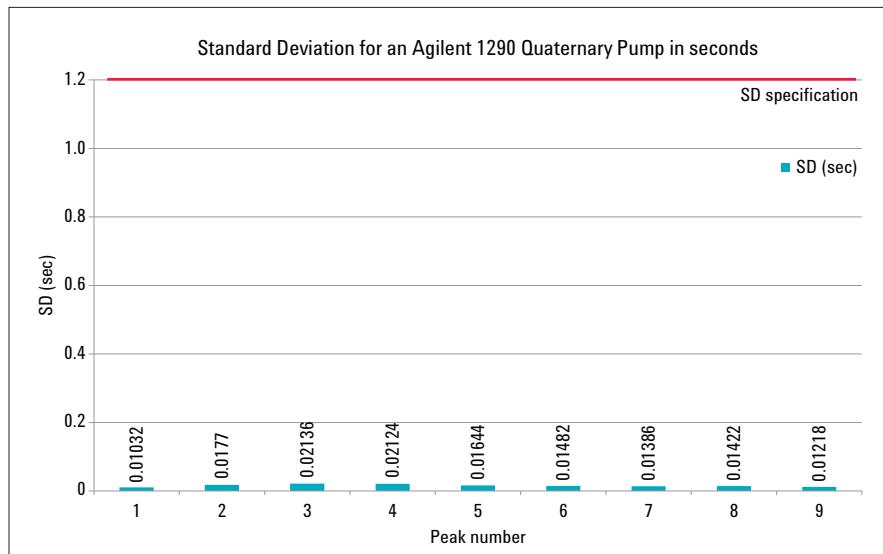

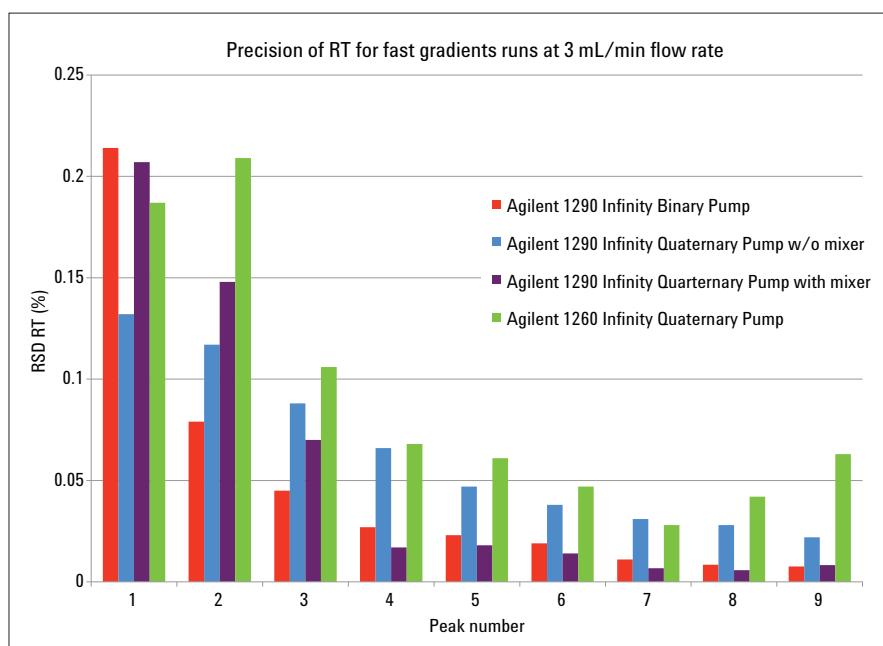**Figure 9**
Precision of retention times for high flow rates.

Figure 10
Standard deviation of retention times.

Experiment 5

A conventional application on a narrow bore column was done using the Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 100 mm, 1.8 µm applying a flow rate of 0.3 mL/min. The 1290 Infinity Quaternary Pump is well suited for applications on long narrow bore columns at moderate flow rates. This type of application is frequently used in combination with mass spectrometry. Figure 11 shows an overlay of the obtained chromatograms. The delay volume was influencing the peak elution time, but for applications, where resolution, not speed, is the first criteria, this is less important.

The 1290 Infinity Quaternary LC showed comparable results with the 1290 Infinity Binary LC, for the applied chromatographic conditions, see Figure 12.

The 1260 Infinity Quaternary LC configuration showed results which were partially above the specification of the 1290 Infinity Quaternary Pump.

The relative standard deviation range of the retention times for the 1290 Infinity Quaternary Pump was 0.116 to 0.014% RSD with mixer and without mixer 0.084 to 0.018% RSD. The range for the 1290 Infinity Binary Pump was 0.091 to 0.011% RSD and the range for the 1260 Infinity Quaternary Pump was 0.291 to 0.075% RSD.
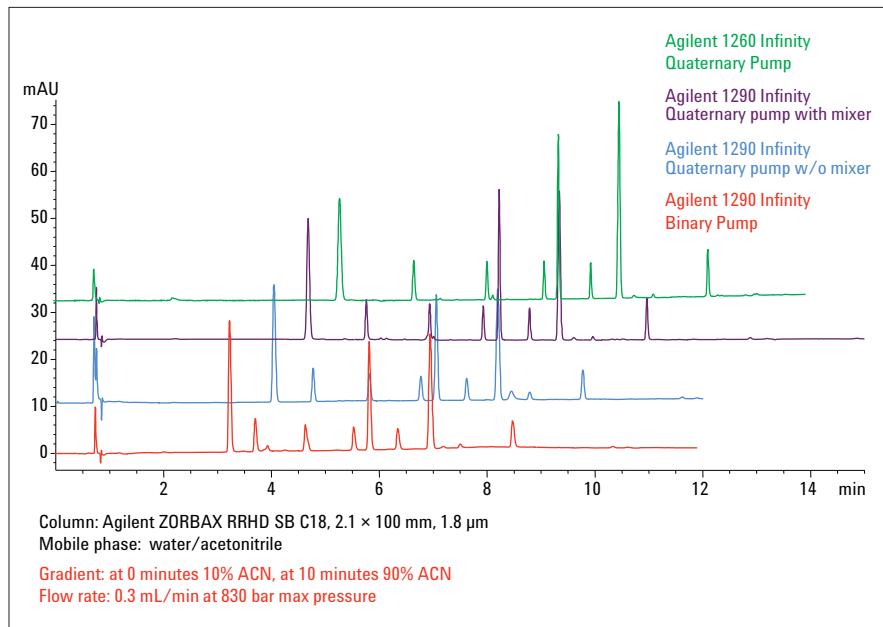

Column: Agilent ZORBAX RRHD SB C18, 2.1 × 100 mm, 1.8 µm
Mobile phase: water/acetonitrile
Gradient: at 0 minutes 10% ACN, at 10 minutes 90% ACN
Flow rate: 0.3 mL/min at 830 bar max pressure

Figure 11
Overlay of chromatograms using a narrow bore column and 0.3 mL/min flow rate, compounds, see Figure 4.
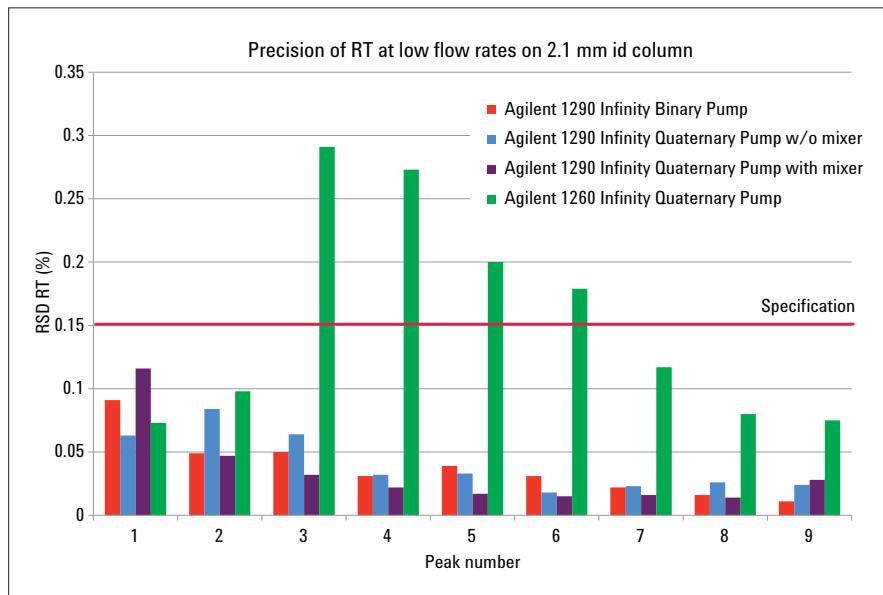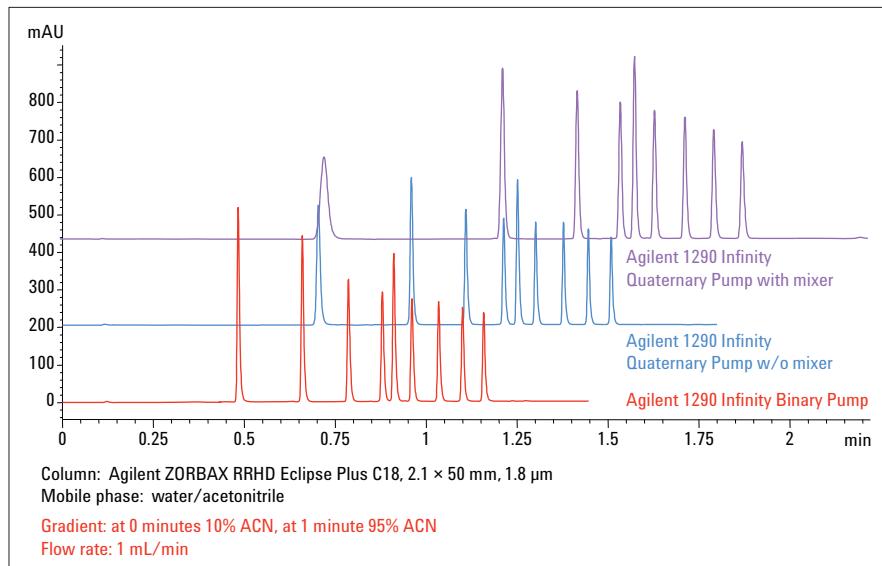

Figure 12
Precision of retention times using a narrow bore column.
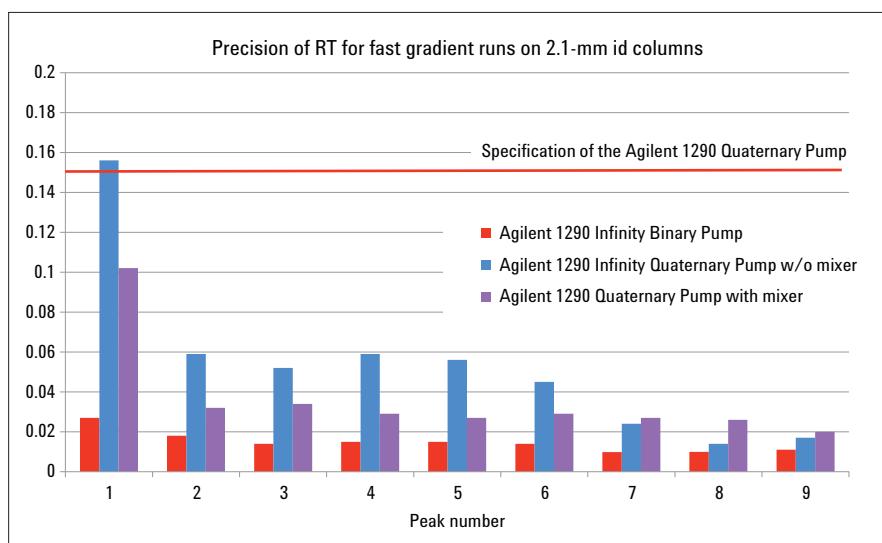
Experiment 6

Fast runs on 2.1 x 50-mm column apply-ing 1.2 mL/min flow rate proved the usability of the 1290 Infinity Quaternary Pump for fast measurements on narrow bore columns, which are frequently used for screening in connection with mass spectrometry. The delay volume influenced the elution times signifi-cantly, see Figure 13. The 1290 Infinity Binary System with its delay volume of only 170 μL delivered all peaks within 1.25 minutes. The same conditions applied on the 1290 Infinity Quaternary System caused an increase of the total run time of 0.35 minutes due to the delay volume of 420 μL. Typically, the 1290 Infinity Binary Pump is best suited for all gradients or isocratic applica-tions using narrow bore columns applying high flow rates with short run times.

The precision of retention times was excellent for both systems, see Figure 14. The standard deviation for the first peak of the 1290 Infinity Quaternary Pump, for example, was as low as 0.000877 minutes (0.053 sec-onds), which is far below the specifica-tion. This shows the excellent relative standard deviations values shown in Figure 14.

The relative standard deviation range of the retention times for the 1290 Infinity Quaternary Pump with mixer was 0.102 to 0.02% RSD, and without mixer 0.156 to 0.017% RSD. The range for the 1290 Infinity Binary Pump was 0.024 to 0.01% RSD. This shows the outstanding performance of the 1290 Infinity Binary Pump.

Column: Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 μm
Mobile phase: water/acetonitrile
Gradient: at 0 minutes 10% ACN, at 1 minute 95% ACN
Flow rate: 1 mL/min

Figure 13
Comparison of chromatograms obtained on the Agilent 1290 Infinity Binary and Quaternary LC Systems applying fast chromatographic conditions using short 2.1-mm id columns, compounds, see Figure 8.

Figure 14
Precision of retention times for fast runs using 2.1-mm id columns.

Conclusions

The Agilent 1290 Infinity Quaternary Pump provided excellent performance with respect to precision of retention times. Excellent results were obtained for conventional gradient and isocratic chromatographic conditions, for fast gradient conditions on 3-mm id columns and 3 mL/min flow rate and for applications using narrow bore columns using conventional conditions. For these applications, the 1290 Infinity Quaternary Pump was comparable to the 1290 Infinity Binary Pump.

For fast chromatographic conditions, applying high flow rates on short 2.1-mm id columns the 1290 Infinity Quaternary Pump showed excellent results with respect to precision of retention times, however, the 1290 Infinity Binary Pump showed exceptional results with respect to elution times as well as precision of retention times.

The applied step gradients showed excellent accuracy and precision over the complete gradient range from 0 to 100% tracer.

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