Scope of low and high pressure mixing

Comparing the Agilent 1260 Infinity Quaternary and Binary Pumps

Technical Overview

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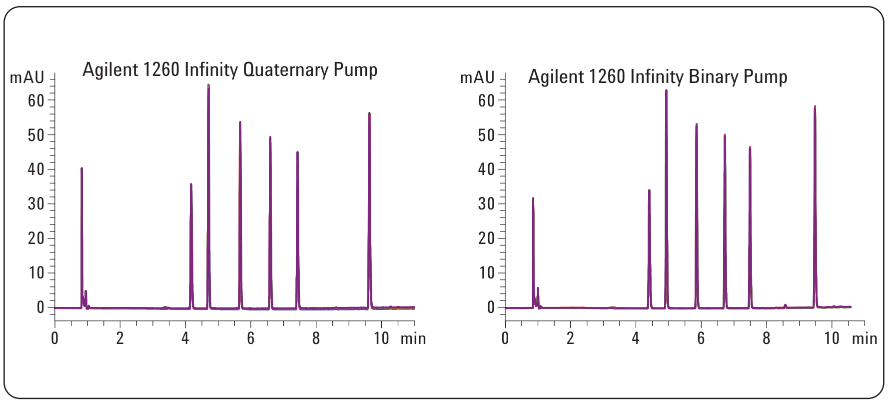

Abstract

The Agilent 1260 Infinity LC family offers two analytical scale LC gradient pump types: a quaternary low pressure mixing pump and a binary high pressure mixing pump. Both pumps show excellent performance within their design range. The optimum gradient range for the quaternary pump is between 5% to 95% organic. The binary pump shows optimum performance from 0% to 100% organic, which is advantageous for compounds eluting at very low or at very high organic percentage. In the range of 5% to 95% organic both pumps show comparable performance. Another advantage for the binary pump is the high mixing efficiency, which is necessary if TFA is used as a modifier. At high flow rates and fast gradients, both pumps show excellent retention time precision. The quaternary pump has the advantage that ternary and quaternary gradients can be applied.

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Introduction

One of the most important performance criteria for a pump is precise retention times. The precision of retention time for gradient runs and isocratic runs where the solvent compositions is formed out of two solvent channels depends mainly on:

- Flow precision
- Mixing performance
- Compressibility settings
- Stroke volumes
- Primary channel selection (Agilent 1260 Infinity Quaternary Pump only)
- Composition accuracy and precision

Two main pump types are typically used in analytical LC: low pressure gradient pumps and high pressure gradients pumps.

In this Application Note, we will discuss the application range of the two pump types comparing the Agilent 1260 Infinity Binary Pump to the Agilent 1260 Infinity Quaternary Pump.

Experimental

An Agilent 1260 Infinity LC system was used including:

- Agilent 1260 Infinity Quaternary Pump (G1311B)
- Agilent 1260 Infinity Binary Pump (G1312B) and Degasser (G1379B)
- Agilent 1260 Infinity Autosampler (G1367E)
- Agilent 1260 Infinity Thermostatted Column Compartments (G1316C)
- Agilent 1260 Infinity Diode Array Detector (G4212B) with 10 mm flow cell
- Several Agilent ZORBAX columns
- Agilent ChemStation B.04.02

Results and discussion

The Agilent 1260 Infinity Binary and Quaternary Pumps are designed for specific application ranges. The Agilent 1260 Infinity Quaternary Pump is the "work horse" for applications using isocratic and conventional gradient conditions, and a practical flow rate range from 0.3 up to 10 mL/min. The Agilent 1260 Infinity Binary Pump is typically used for more demanding applications, especially for gradients from 0% to 10%, 90% to 100% and a practical flow rate range from 0.05 to 5 mL/min. This pump is the best choice for TFA as a modifier in the mobile phase because of its excellent mixing performance.

Table 1 presents an overview of the important parameters and specifications for the two pump types.

Some specific pump parameters must be set by the user and the settings have an effect on pump performance:

- The **compressibility** (see Appendix), which influences the flow accuracy over the complete pressure range, can be set to its optimum for each solvent channel using the binary pump. For the quaternary pump only one value can be entered for all channels, which can cause lower retention time precision.
- The **stroke volume** (see Appendix) setting influences the mixing performance. As the flow rate decreases, the stroke volume should decrease as well. Typically the AUTO setting is appropriate for both pumps.

The following experiments verified the performance and demonstrated the differences between the two pumps. Retention time precision was measured for the following conditions:

- approximately 50% acetonitrile/50% aqueous
- slow generic gradient using MeOH/water
- slow generic gradient using ACN/water and TFA as modifier
- low organic percentage
- high organic percentage
- 3 mL/min flow rate and gradient time of 1 min

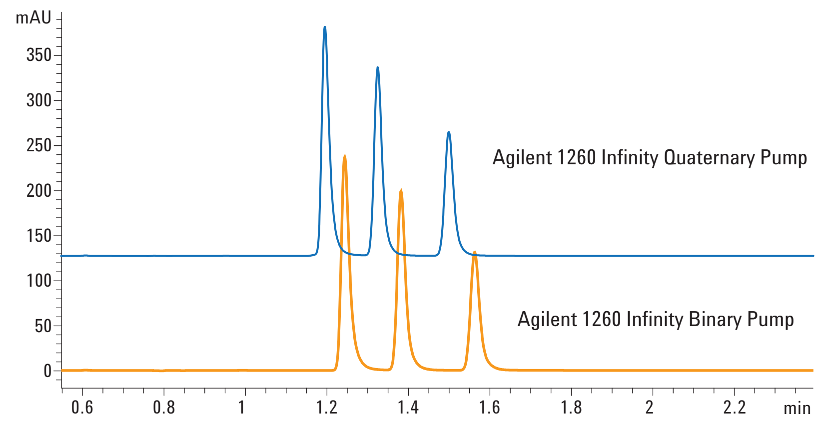
Agilent 1260 Infinty Quaternary Pump Primary channel = A (default)	**Agilent 1260 Infinity Binary Pump**
Low pressure gradient pump	High pressure gradient pump with standard and low delay volume configuration
One compressibility setting for all used mobile phases	Compressibility setting individually related to the selected mobile phase
Maximum flow rate is 10 mL/min Up to 600 bar for 5 mL/min	Maximum flow rate is 5 mL/min up to 600 bar
Ternary and quaternary gradients	Binary gradient
Flow precision: ≤ 0.07% RSD*	Flow precision: ≤ 0.07% RSD*
Composition precision: < 0.2% RSD*	Composition precision: < 0.15% RSD*
Optimum gradient range: 5% to 95%	Optimum gradient range: 0% to 100%

Table 1
Design and specifications
*measured for retention times under conditions described in the manuals

Retention time precision at approximately 50% acetonitrile/50% aqueous

For most pumps the mixing performance at approximately 50% organic and 50% aqueous is a critical performance point. Experimental conditions were selected to optimize performance of both types and the analgesics antipyrine, phenobarbital, and phenacetin were analyzed (Figure1). The mobile phases were mixed out of two channels. The different designs of high and low pressure mixing pumps produce different mixing compositions, which result in different retention times for both pumps. The quaternary pump adds slightly more organic phase to the composition mixture resulting in shorter retention times.

The results are presented in Figure 2. The binary pump shows slightly better results for the first two peaks, but overall the results are comparable.



Chromatographic conditions

Compounds:	Antipyrine, phenobarbital, phenacetin
Concentration:	0.3 ng/µL each
Column:	Agilent ZORBAX SB C8 RRHT, 100 mm × 4.6 mm, 1.8 µm
Mobile phases:	Water/acetonitrile = 55/45, isocratic from two channels
Flow rate:	1.2 mL/min
Stop time:	5 min
Column temperature:	40 °C
Injection volume:	5 µL
DAD:	220/10 nm Ref. 360/100, 20 Hz

Figure 1
Analysis of analgesics using isocratic conditions and 55/45 water/ACN mixture.

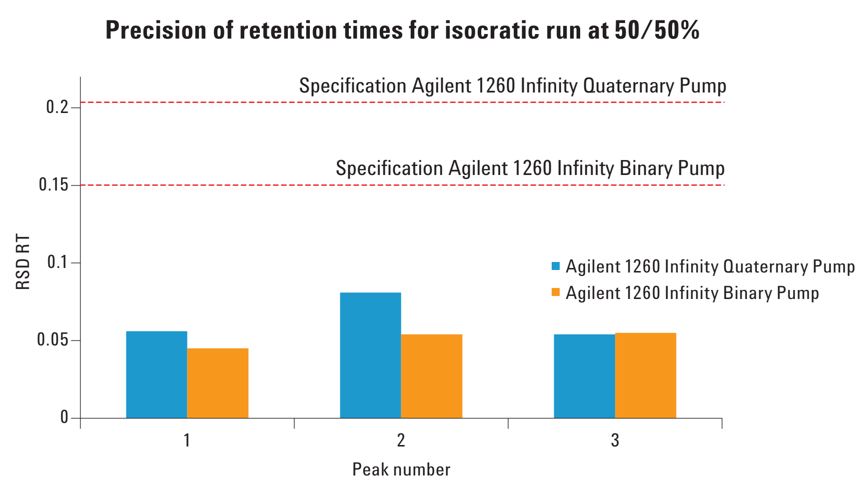


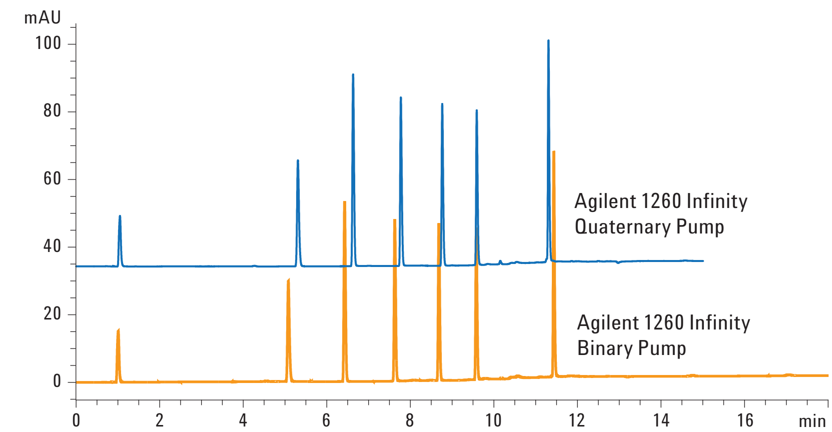
Figure 2
Comparable precision of retention times using isocratic conditions.

Retention time precision for a conventional generic gradient using MeOH/water as eluents

Both pumps typically show comparable results for conventional gradients from about 5% to 95% in 10 min. Mixing performance and pump ripple do not differ significantly. The experiments were done using a paraben standard (Figure 3).

The delay volume of an LC system determines how fast a gradient change can reach the head of the column in gradient applications. Typically high pressure mixing pumps have smaller delay volumes than low pressure mixing systems. This is also true for the delay volumes of the Agilent 1260 Infinity Quaternary and Binary Pumps. A peak analyzed with the Agilent 1260 Infinity Binary Pump elutes earlier than on the Agilent 1260 Infinity Quaternary Pump.

The results for both pumps are shown in Figure 4. As expected, both pumps showed excellent and comparable results with acetonitrile as mobile phase (data not shown).



Chromatographic conditions

Compounds:	Uracil, phenol, methyl-, ethyl-, propyl-, butyl- and heptylparaben
Column:	Agilent ZORBAX Eclipse C18 RRHT, 100 mm × 4.6 mm, 1.8 µm
Mobile phases:	Water/methanol
Gradient:	10% to 90% in 10 min
Flow rate:	1.2 mL/min
Stop time:	18 min
Column temperature:	40 °C
Injection volume:	3 µL
DAD:	254/10 nm Ref. 360/100 nm, 20 Hz

Figure 3
Analysis of paraben sample using a conventional gradient from 10% to 90% organic (methanol) in 10 min.

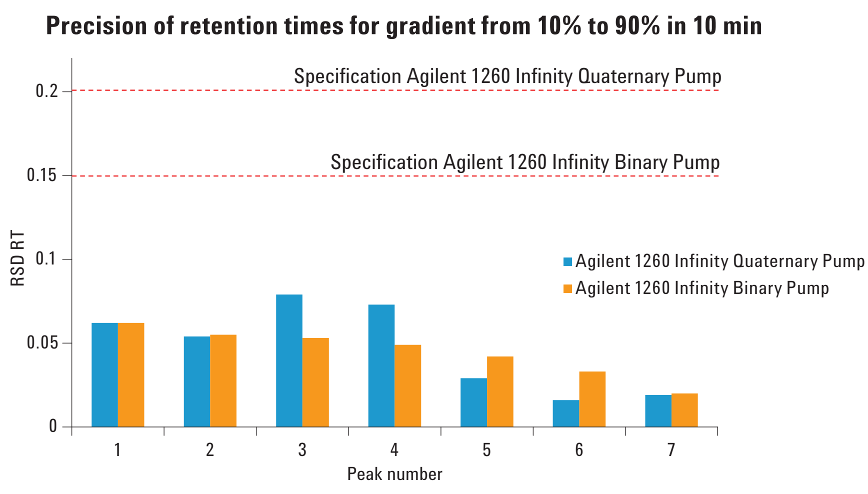


Figure 4
Comparable precision of retention times for conventional gradients using methanol as organic phase.

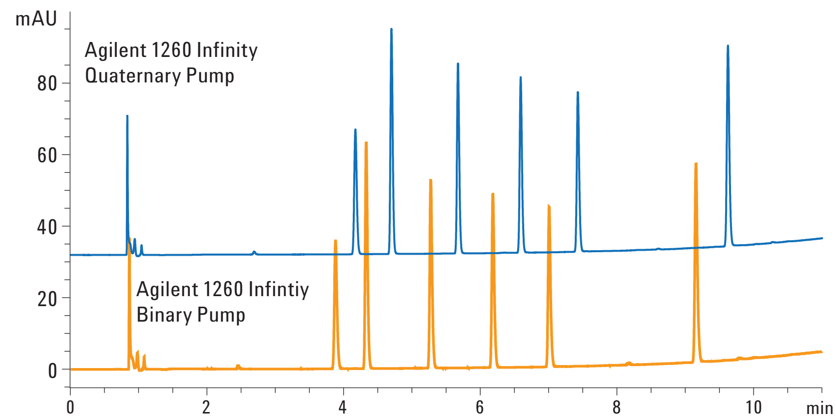
Retention time precision for conventional generic gradient using ACN/water and TFA as the modifier

In the following experiment, TFA was added to both mobile phases. TFA is known to cause problems for the precision of retention times if mixing of the aqueous and organic phases during a gradient run is not sufficient.

For the binary pump, mixing of the mobile phases takes place at the high pressure side using a 420-µL mixer, where turbulences occur due to the packing material. This provides very effective mixing and reduces the TFA problems significantly. For the quaternary pump, mixing occurs in pump heads and capillary connections. Typically mixing is less effective and the precision of retention times might be worse.

In Figure 5 the chromatograms obtained using the binary and quaternary pump are overlaid. TFA was added at 0.05% to the aqueous and the organic (acetonitrile) phase.

In Figure 6, the results show that the binary pump produces slightly better retention time precision due to the higher mixing performance.



Chromatographic conditions

Compounds:	Uracil, phenol, methyl-, ethyl-, propyl-, butyl- and heptylparaben
Column:	Agilent ZORBAX Eclipse Plus C18 RRHT, 100 mm × 4.6 mm, 1.8 µm
Mobile phases:	Water/acetonitrile + 0.05% TFA each
Gradient:	10% to 90% in 10 min
Flow rate:	1.2 mL/min
Stop time:	11 min
Post time:	4 min
Column temperature:	40 °C
Injection volume:	3 µL
DAD:	254/10 nm Ref. 360/100 nm, 20 Hz

Figure 5
Analysis of the paraben sample using TFA as modifier in both mobile phases.

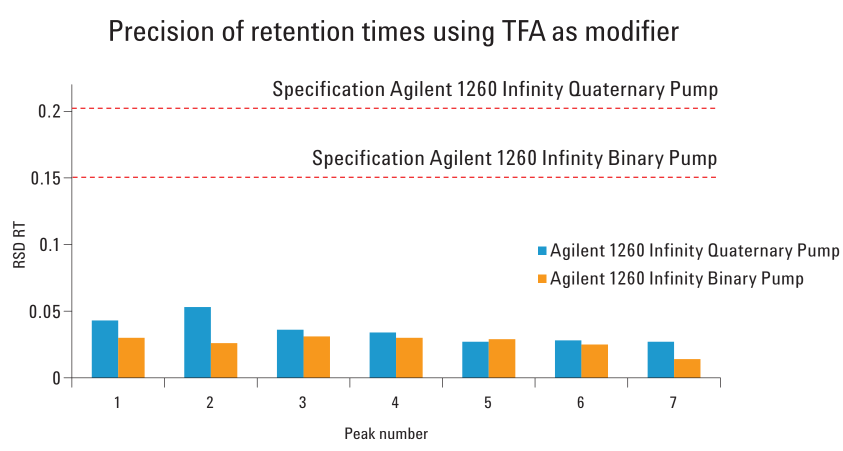


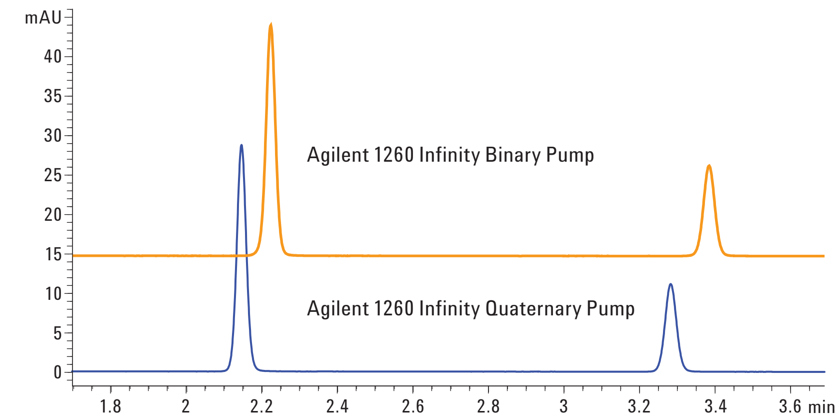
Figure 6
Better precision of retention times for the binary pump using TFA as modifier.

Retention time precision at low organic percentage

In this experiment, the precision of retention time was tested running a gradient from 2% to 12% organic (Figure 7). During the isocratic run, the quaternary pump adds slightly more organic phase to the mixture resulting in earlier elution.

In this low organic percentage range the use of the binary pump is advantageous (Figure 8). The difference in precision is significant for the early eluting peak (theophylline), which elutes between 4% and 5% organic. The caffeine, which elutes at higher organic percentage, shows similar precision on both pumps.

Although the range of 0% to 5% organic phase is not specified for the quaternary pump, the RSD for retention times is still below specification.



Chromatographic conditions

Compounds:	Theophylline and caffeine
Column:	Agilent Poroshell 120 EC 18, 50 mm × 4.6 mm, 2.7 µm
Mobile phases:	Water/acetonitrile
Gradient:	2% to 12% ACN in 5 min
Flow rate:	2 mL/min
Stop time:	5 min
Post time:	4 min
Column temperature:	40 °C
Injection volume:	5 µL
DAD:	254/10 nm Ref. 360/100 nm, 20 Hz

Figure 7
Analysis of theophylline and caffeine at low organic percentage.

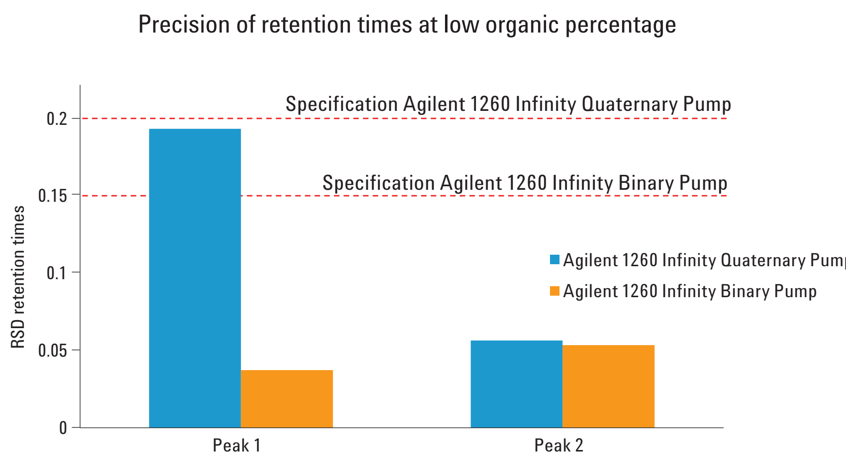
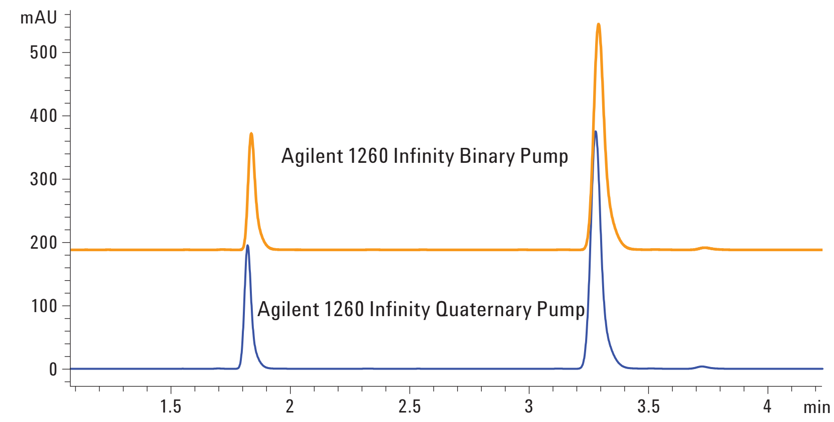


Figure 8
Better results for the binary pump at low organic percentage.

Retention time precision at high organic percentage

In this experiment Sudan Red 1 and Sudan Red 3 were analyzed using a gradient of 90% to 100% organic (Figure 9). The results show that at the highest organic percentage the binary pump provides three to four times better precision (Figure 10).



Chromatographic conditions

Compounds:	Sudan Red 1(50 ng/µL) and 3 (300 ng/µL)
Column:	Agilent ZORBAX Eclipse Plus C18 RRHT, 100 mm × 4.6 mm, 1.8 µm
Mobile phases:	Water/acetonitrile
Gradient:	90% to 100% in 5 min
Flow rate:	1.2 mL/min
Stop time:	6 min
Post time:	4 min
Column temperature:	40 °C
Injection volume:	3 µL
DAD:	490/20 nm Ref. off, 20 Hz
Primary channel for quanternary pump:	A

Figure 9
Analysis of Sudan Red 1 and 3 at high organic percentage.

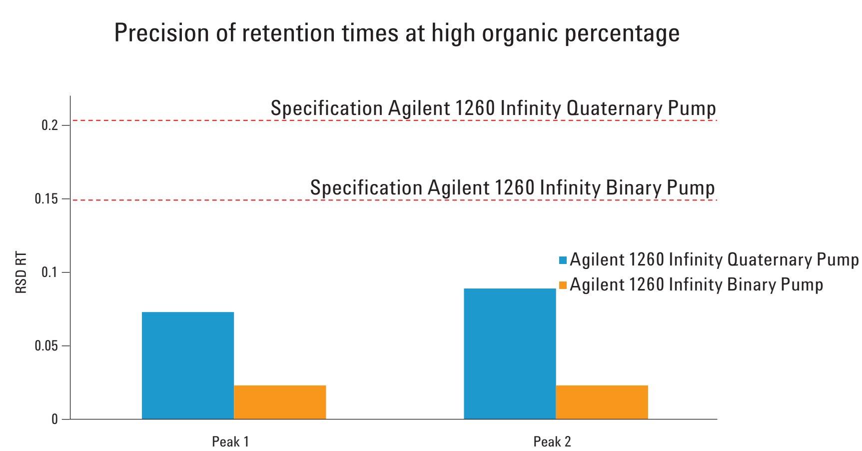
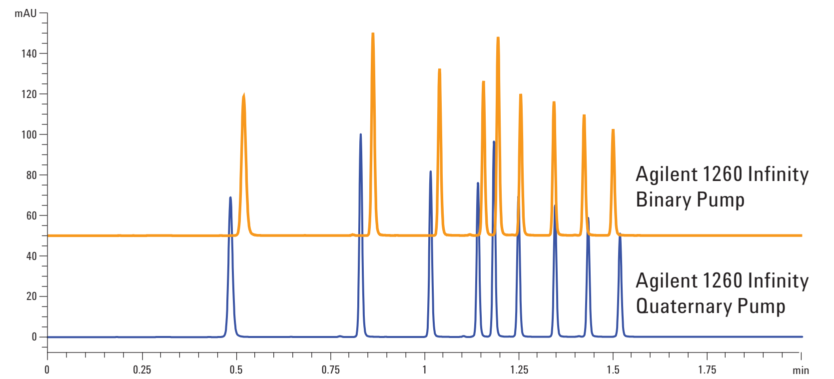


Figure 10
Better results for the binary pump at high organic percentage.

Retention time precision at 3 mL/min flow rate and a gradient time of 1 min

Sufficient resolution in the shortest possible run time is important in chromatography. This means that short columns, high flow rates, and short gradient times are applied. Chromatographic conditions were selected to demonstrate that the Agilent 1260 Infinity Quaternary and Binary Pumps can provide optimum performance. A Poroshell 120 50 mm × 4.6 mm column was selected and operated at a flow rate of 3 mL/min. Gradient time was set to 1 min. Using these conditions the first peaks elute at approximately 0.5 min and the last one at approximately 1.5 min (Figure 11).

The results are presented in Figure 12 and show that both pumps perform well. The precision for retention times is < 0.05% RSD overall.



Chromatographic conditions

Compounds:	RRLC checkout sample (p/n 5188-6529)
Column:	Agilent Poroshell 120 EC C18, 50 mm × 4.6 mm, 2.7 µm
Mobile phases:	Water/acetonitrile
Gradient:	20% to 90% in 1 min
Flow rate:	3 mL/min
Stop time:	2 min
Post time:	1 min
Column temperature:	40 °C
Injection volume:	3 µL
DAD:	245/10 nm Ref. 360/100 nm, 20 Hz

Figure11
Analysis of phenone sample using high flow rates.

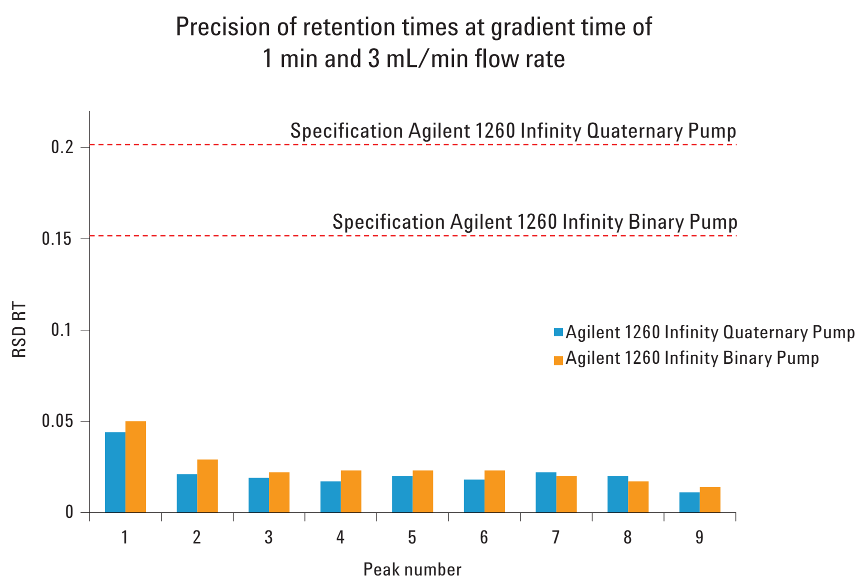


Figure 12
Comparable precision of retention times applying fast chromatographic conditions.

Conclusion

The performances of the Agilent 1260 Infinity Binary and Quaternary Pumps were tested and compared using several different chromatographic conditions. These conditions include isocratic runs and conventional gradients with and without TFA as modifier; low and high organic percentage; and fast chromatographic conditions. The results show that the binary pump has advantages if the mixing performance is demanding, such as when TFA is used as modifier. The retention time precision was compared in the ranges of 0% to 10% organic and 90% to 100% organic and showed that the performance of the binary pump is better. For conventional gradients between 5% and 95% organic and isocratic runs, the performance of both pumps is comparable. Results are also comparable for high flow rates and short gradient times in the range of 10% to 90% organic.

Appendix

Compressibility

When a solvent is metered at ambient pressure and compressed to a higher pressure, the volume decreases. This is due to an effect known as solvent compressibility. Solvent compressibility is a nonlinear function of pressure and temperature. It is unique to every solvent. In order to deliver the desired flow accurately at all pressures, Agilent pumps use compressibility compensation. Usually, an average compressibility value for the solvent is used across the whole pressure range of the pump, such as that applied for the quaternary pump. The Agilent 1260 Infinity Binary Pump has a different compressibility compensation concept. The compressibility of a solvent is determined at different pressures between 0 to 600 bar. This pump uses the obtained nonlinear function to select the correct compressibility value for the actual pump pressure. Compressibility data for the most common solvents are readily available in the pump firmware.

Stroke Volume

The smaller the solvent volume in the pump chamber, the faster it can be recompressed to operating pressure. The Agilent 1260 Infinity pumps allow manual or automatic adjustment of the pump stroke volume of the first piston in the range of 20 – 100 µL. Due to the compression of the solvent volume in the first pump chamber, each piston stroke of the pump will generate a small pressure pulsation, influencing the flow ripple of the pump. The amplitude of the pressure pulsation is mainly dependent on the stroke volume and the compressibility compensation for the solvent in use. Small stroke volumes generate less pressure pulsation than larger stroke volumes at the same flow rate. In addition, the frequency of the pressure pulsation is higher. This decreases the influence of flow pulsations on quantitative results. In gradient mode, smaller stroke volume results in less flow ripple and improves the composition ripple. The Agilent pumps use a processor-controlled ball screw system to drive its pistons. The normal stroke volume is optimized for the selected flow rate. Small flow rates use a small stroke volume while higher flow rates use a higher stroke volume. The stroke volume for the pump is by default set to AUTO mode. This means that the stroke is optimized for the flow rate in use. A change to larger stroke volumes is possible but not recommended.

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