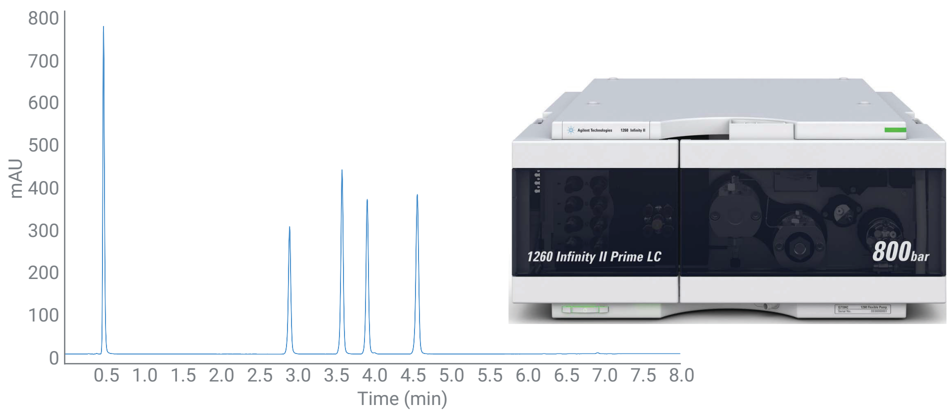
Technical Overview

The Agilent 1260 Infinity II Flexible Pump

The Benchmark for High Accuracy and Precision in Low Pressure Mixing Gradient Pumps



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Abstract

The Agilent 1260 Infinity II Flexible Pump provides the highest flexibility for running conventional applications on 4.6 mm columns or fast runs on 2.1 and 3.0 mm id columns with no compromise regarding precision and accuracy.

This Technical Overview demonstrates the outstanding performance of the 1260 Infinity II Flexible Pump. Precision of retention times under demanding conditions was evaluated and compared to the Agilent 1260 Infinity II Binary Pump. In addition, the composition accuracy, delay volume, and flow accuracy of both pumps were evaluated by running step gradients.

Introduction

The Agilent 1260 Infinity II Flexible Pump is a low-pressure mixing gradient pump, and is capable of 800 bar operating pressure. The integrated multipurpose valve enables convenient features such as automatic purge, switching an additional mixer in and out, and filter backflush.

To demonstrate the superior performance of the 1260 Infinity II Flexible Pump, different demanding applications were chosen. Results obtained with the 1260 Infinity II Flexible Pump were compared to the results obtained with the Agilent 1260 Infinity II Binary Pump.

Experimental

Instrumentation

For the experiments, the following modules were used:

- Agilent 1260 Infinity II Flexible Pump (G7104C)
- Agilent 1260 Infinity II Binary Pump (G7112B)
- Agilent 1260 Infinity II Multisampler (G7167A), equipped with an integrated sample cooler (option #100)
- Agilent 1260 Infinity II MCT (G7116A)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A), equipped with a standard flow cell: 10 mm path length,13 µL volume

Software

Agilent OpenLAB CDS 2.2 (M8413A)

Chemicals

All solvents were LC grade. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a 0.22 µm membrane point-of-use cartridge (Millipak). Acetonitrile and acetone were purchased from Merck (Darmstadt, Germany). Trifluoroacetic acid (TFA) and formic acid (FA) were purchased from Sigma-Aldrich (Steinheim, Germany).

Methods

Table 1. Chromatographic conditions for step gradients.

Parameter	Value
Column	Restriction capillary
Solvent	A) 0.3 % Acetone in water B) Water
Gradient	Between 20 and 45 minutes in 1 % steps from 100 to 94 %A Between 45 and 55 minutes in 2 % steps from 94 to 90 %A Between 55 and 80 minutes in 10 % steps from 90 to 51 %A Between 80 and 90 minutes in 1 % steps from 51 to 49 %A Between 90.01 and 110 minutes in 10 % steps from 40 to 10 % Between 110.01 and 135 minutes in 2 % steps from 6 to 0 %A
Stop time	140 minutes
Flow rate	1 mL/min
Column temperature	30 °C
DAD	273/4 nm, Ref. 360/100 nm, 10 Hz

Table 2. Chromatographic conditions for shallow gradient runs.

Parameter	Value
Compounds	Polyphenolic mix: Catechin, caffeic acid, epicatechin, epigallocatechin (all purchased from Sigma-Aldrich)
Column	Agilent ZORBAX StableBond C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Solvent	A) 0.1 % FA in water B) 0.1 % FA in acetonitrile
Gradient	0 minutes–6 %B 8 minutes–12 %B
Stop time	8 minutes
Post time	3 minutes
Flow rate	0.6 mL/min
Injection volume	2 µL with 3 seconds needle wash (60 % acetonitrile in water)
Sample temperature	10 °C
Column temperature	30 °C
DAD	280/4 nm, Ref. 360/50 nm, 40 Hz

Table 3. Chromatographic conditions for low organic percentage runs.

Parameter	Value
Compounds	Sulfa drugs: Sulfanilamid, sulfathiazole, sulfamerazine, sulfachloropyridazine, sulfamethazine (all purchased from Sigma-Aldrich)
Column	Agilent ZORBAX StableBond C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Solvent	A) 0.1 % TFA in water B) 0.1 % TFA in acetonitrile
Gradient	0 minutes–1 %B 6 minutes–25 %B
Stop time	8 minutes
Post time	3 minutes
Flow rate	0.6 mL/min
Injection volume	2 µL with 3 seconds needle wash (60 % acetonitrile in water)
Sample temperature	10 °C
Column temperature	45 °C
DAD	254/4 nm, Ref. 360/80 nm, 20 Hz

Results and Discussion

Experiment 1: Step gradient

Step gradients, including a tracer in solvent A, were used to determine the system delay volume, the composition accuracy, mixing noise, and flow accuracy. For the flow accuracy evaluation, a flow accuracy meter (FAM) was used.

Step gradients under identical conditions were applied to the 1260 Infinity II Flexible Pump, with and without the Jet Weaver mixer, and to the 1260 Infinity II Binary Pump. Figure 1 shows an overlay of the three step gradients running from 100 %A (water + tracer) to 0 %A (water + tracer) in 1, 2, and 10 % steps. Both pumps showed excellent performance over the complete gradient range. The gradient steps were perfectly resolved even for the 1 % steps at low % solvent B. These results clearly demonstrate an equivalent gradient performance of the 1260 Infinity II Flexible Pump compared to the 1260 Infinity II Binary Pump, even for demanding ranges like 1 to 5 %B.

Table 4. Chromatographic conditions for high organic percentage runs.

Parameter	Value
Compounds	Sudan red mix: Sudan I, Sudan II, Sudan III, Sudan IV (all purchased from Sigma-Aldrich)
Column	Agilent ZORBAX Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959941-302)
Solvent	A) Water B) 95 % Acetonitrile in water
Gradient	0 minutes–90 %B 4 minutes – 100 %B
Stop time	4 minutes
Post time	2 minutes
Flow rate	1 mL/min
Injection volume	1 µL with 3 seconds needle wash (acetonitrile)
Sample temperature	10 °C
Column temperature	40 °C
DAD	490/20 nm, no Ref., 20 Hz

Table 5. Chromatographic conditions for fast gradient runs.

Parameter	Value
Compounds	Agilent RRLC checkout sample (p/n 5188-6529)
Column	Agilent ZORBAX Eclipse Plus C18, 3.0 × 50 mm, 1.8 µm (p/n 959941-302)
Solvent	A) Water B) Acetonitrile
Gradient	0 minutes–30 %B 1 minutes–100 %B
Stop time	2 minutes
Post time	1 minutes
Flow rate	2 mL/min
Injection volume	1 µL with 3 seconds needle wash (60 % acetonitrile in water)
Sample temperature	10 °C
Column temperature	60 °C
DAD	254/4 nm, Ref. 360/60, 80 Hz

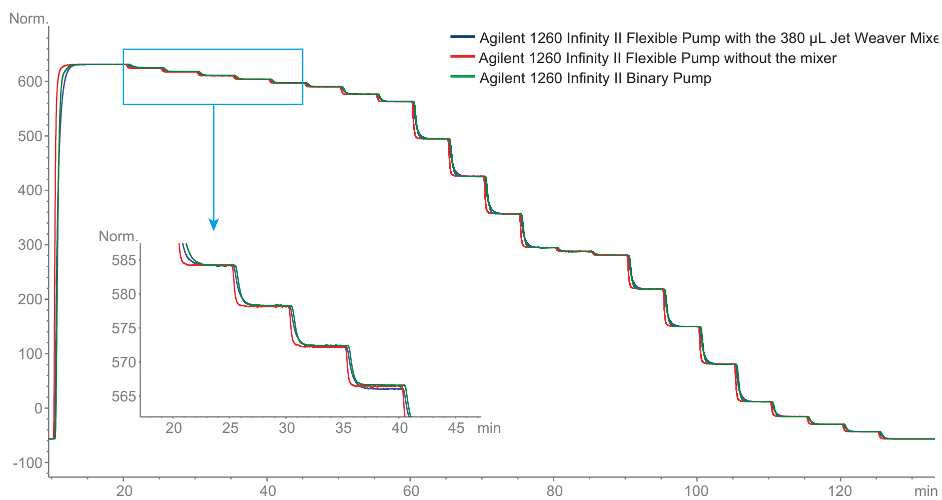

Figure 1. Step gradients from 100 to 0 % tracer on an Agilent 1260 Infinity II Flexible Pump with and without a mixer, and on an Agilent 1260 Infinity II Binary Pump.

Composition accuracy was calculated for both pumps based on the step gradients. Figure 2 shows the composition accuracy for the 1260 Infinity II Flexible Pump (with and without the 380 µL Jet Weaver mixer) and for the 1260 Infinity II Binary Pump. The specifications for composition accuracy were defined as ±0.35 % for the 1260 Infinity II Binary Pump and ±0.4 % for the 1260 Infinity II Flexible Pump. The composition accuracy for both pumps was inside the specified range.

Table 7 summarizes the system delay volume, mixing noise, and flow accuracy. As expected, the 1260 Infinity II Flexible Pump without the Jet Weaver mixer had the lowest delay volume, approximately 310 µL. Switching the 380 µL Jet Weaver mixer into the flowpath increased the delay volume to only 460 µL. For the 1260 Infinity II Binary Pump, a delay volume of 610 µL was calculated. All pump delay volumes include the volume of the capillaries, restriction capillary, and detector cell. The 1260 Infinity II Multisampler was not part of the flowpath for these measurements.

The mixing noise was calculated for every step over the complete gradient. Table 7 compares the values at three different steps: 95 %, 49 %, and 4 % tracer. The 1260 Infinity II Flexible Pump and the 1260 Infinity II Binary Pump showed similar results. However, the 1260 Infinity II Flexible Pump with the 380 µL Jet Weaver mixer provided the best results regarding the mixing noise. With the additional mixer, the delay volume of the 1260 Infinity II Flexible Pump increased slightly, but facilitates a more stable UV signal due to a better mixing performance. These results clearly demonstrate the outstanding performance of the Jet Weaver mixer with efficient mixing and smart design to keep the delay volume as low as possible.

By adding a FAM to the LC stack configuration, the actual flow over the step gradient runs was monitored.

For the 1260 Infinity II Flexible Pump, the real flow rate (0.998 mL/min) was slightly lower than the programmed flow rate of 1 mL/min. The real flow rate for the 1260 Infinity II Binary Pump showed slightly higher values with 1.003 mL/min. However, both pumps performed excellently, and were inside the specification range of ±1 %.

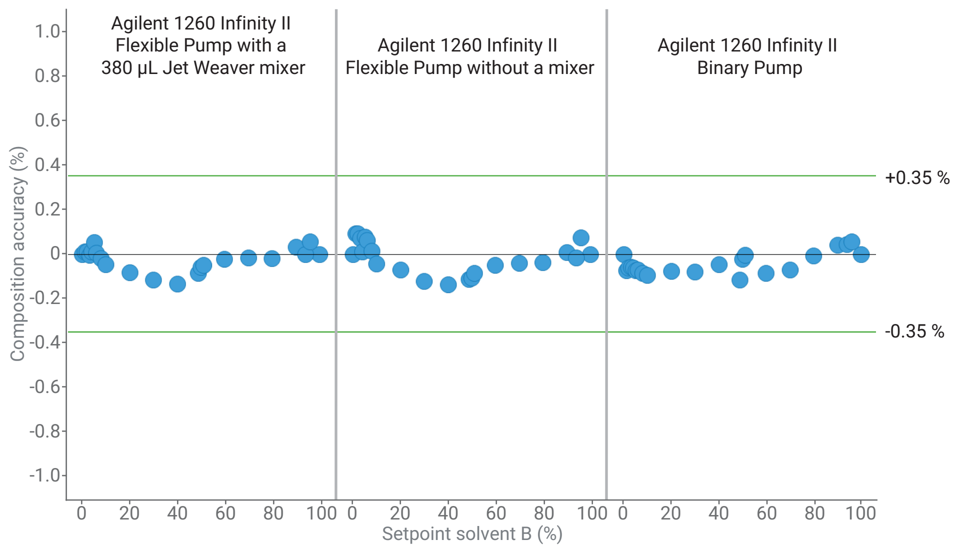

Figure 2. Composition accuracy for the Agilent 1260 Infinity II Flexible Pump with and without a mixer, and for the Agilent 1260 Infinity II Binary Pump.

Table 7. Step gradients results.

	Agilent 1260 Infinity II Flexible Pump with the 380 µL Jet Weaver mixer	Agilent 1260 Infinity II Flexible Pump	Agilent 1260 Infinity II Binary Pump
Delay volume	~460 µL	~310 µL	~610 µL
Mixing noise at 95 %A	0.015 %	0.048 %	0.023 %
Mixing noise at 49 %A	0.024 %	0.040 %	0.045 %
Mixing noise at 4 %A	0.013 %	0.034 %	0.012 %
Mean flow rate (mL/min)	0.998	0.998	1.003
Mean Δ flow rate (%)	-0.164	-0.155	0.270

Experiment 2: Retention time precision for shallow gradients

To determine the retention time precision under shallow gradient conditions, a mix of four polyphenolic compounds was analyzed. The gradient went from 6 %B to 12 %B in eight minutes, representing a change of solvent B of 0.75 % per minute. Figure 3 shows an overlay of two chromatograms, one acquired with the 1260 Infinity II Flexible Pump (without additional mixer) and the other with the 1260 Infinity II Binary Pump. The retention time precisions (Table 8) for the four polyphenolic compounds were similar on both pumps. This experiment demonstrates the high mixing precision of the 1260 Infinity II Flexible Pump even under shallow gradient conditions, where a binary pump is preferred.

Experiment 3: Retention time precision for low organic percentage

A mix of five sulfa drugs was analyzed, running a gradient from 1 to 25 % organic (Figure 4). For this low organic percentage range, a binary pump is advantageous. The 1260 Infinity II Flexible Pump is specified for a composition range of 0 to 100 %. To evaluate the performance at this low organic percentage range for both pumps, the retention time precision was calculated from eight consecutive runs (Table 9).

Both pumps showed excellent performance regarding retention time precision. For the two early eluting compounds sulfanilamide and sulfathiazole, the 1260 Infinity II Flexible Pump even outperformed the 1260 Infinity II Binary Pump.

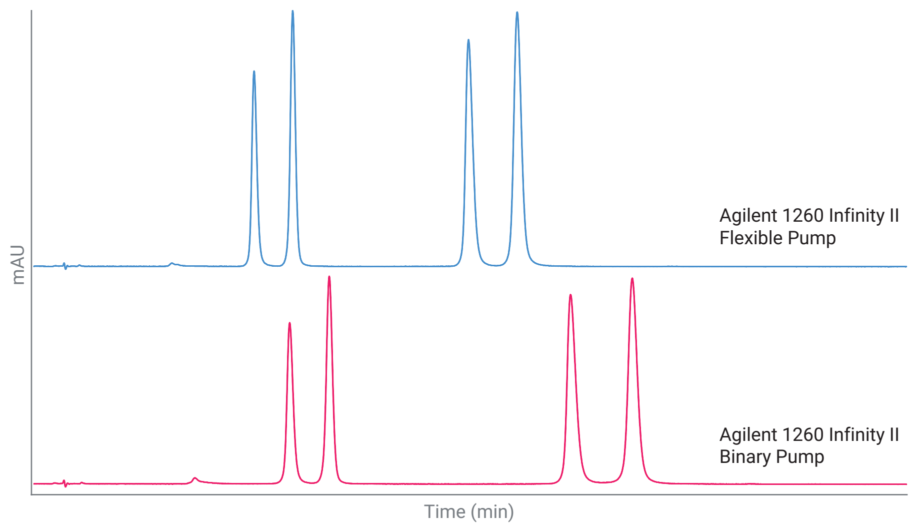

Figure 3. Analysis of polyphenolic compounds using a shallow gradient. Elution order: catechin, caffeic acid, epicatechin, then epigallocatechin.

Table 8. Retention time precision for the analysis of polyphenolic compounds. Eight consecutive runs were used for the calculation.

Agilent 1260 Infinity Flexible Pump				
	Catechin	Caffeic acid	Epicatechin	Epigallocatechin
Average RT (min)	2.019	2.372	3.979	4.426
Standard deviation RT	0.0018	0.0015	0.0042	0.0036
RSD RT	0.087	0.061	0.106	0.081
Agilent 1260 Infinity II Binary Pump				
Average RT (min)	2.348	2.710	4.927	5.491
Standard deviation RT	0.0035	0.0023	0.0032	0.0033
RSD RT	0.151	0.085	0.066	0.060

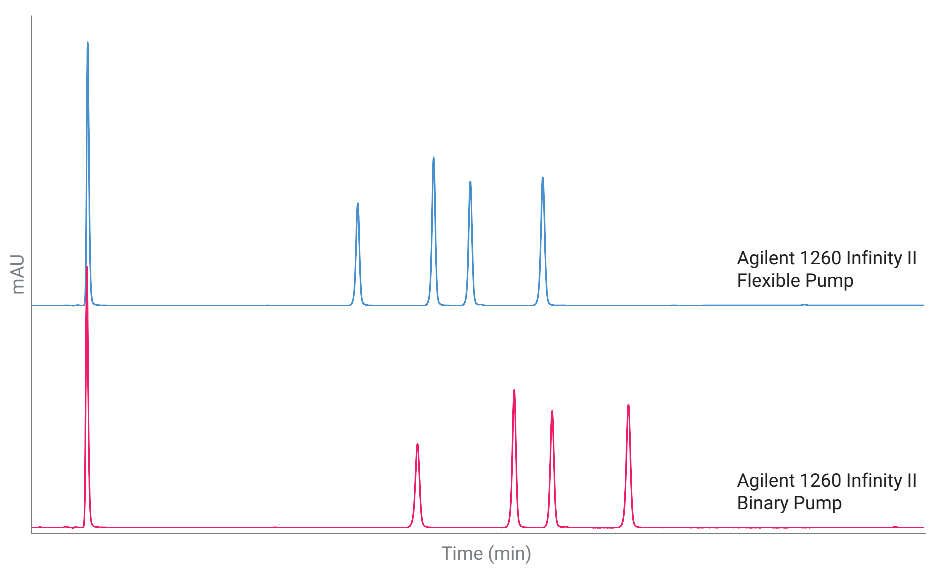

Figure 4. Analysis of sulfa drugs at low organic percentage. Elution order: sulfanilamide, sulfathiazole, sulfamerazine, sulfachloropyridazine, then sulfamethazine.

Experiment 4: Retention time precision for high organic percentage

For this experiment, Sudan Red 1, 2, 3, and 4 were analyzed using a gradient from 90 to 100 % organic (Figure 5 and Table 10). The results show that the 1260 Infinity II Flexible Pump is well suited for this kind of application, and performs better than the 1260 Infinity II Binary Pump.

Table 9. Retention time precision for the analysis of sulfa drugs.

Agilent 1260 Infinity Flexible Pump					
	Sulfanilamid	**Sulfathiazole**	**Sulfamerazine**	**Sulfachloropyridazine**	**Sulfamethazine**
Average RT (min)	0.505	2.926	3.607	3.935	4.586
Standard deviation RT	0.0005	0.0009	0.0012	0.0013	0.0005
RSD RT	0.092	0.032	0.033	0.033	0.010
Agilent 1260 Infinity II Binary Pump					
Average RT (min)	0.499	3.468	4.331	4.671	5.355
Standard deviation RT	0.0009	0.0044	0.0022	0.0022	0.0024
RSD RT	0.174	0.127	0.050	0.047	0.045

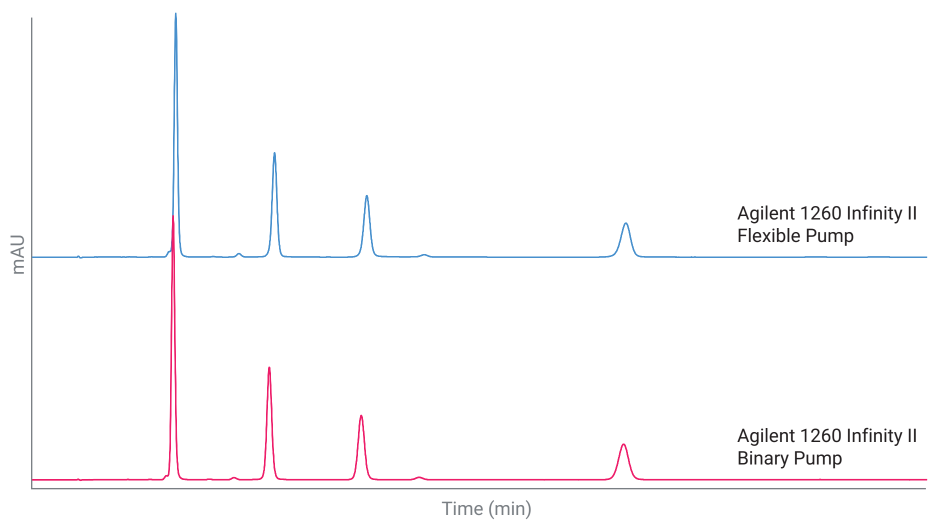

Figure 5. Analysis of a mix of Sudan Red compounds at high organic percentage. Elution order: Sudan Red 1, Sudan Red 2, Sudan Red 3, then Sudan Red 4.

Table 10. Retention time precision for the analysis of Sudan Red compounds. Eight consecutive runs were used for the calculation.

Agilent 1260 Infinity Flexible Pump				
	Sudan I	**Sudan II**	**Sudan III**	**Sudan IV**
Average RT (min)	0.643	1.085	1.497	2.656
Standard deviation RT	0.0004	0.0008	0.0007	0.0014
RSD RT	0.055	0.070	0.047	0.053
Agilent 1260 Infinity II Binary Pump				
Average RT (min)	0.630	1.058	1.468	2.640
Standard deviation RT	0.0009	0.0018	0.0027	0.0044
RSD RT	0.147	0.173	0.187	0.166

Experiment 5: Retention time precision for fast runs

To enable shorter runs, a high flow rate of 2 mL/min was applied. The gradient time was set to one minute, and went from 30 to 100 % organic (Figure 6). For the 1260 Infinity II Binary Pump, the standard configuration and the low delay configuration were tested. For the low delay configuration, the damper and mixer were bypassed. The 1260 Infinity II Flexible Pump was used with and without the mixer. The pressure for all system configurations was approximately 540 bar.

The 1260 Infinity II Binary Pump with the low delay configuration provided the shortest run time, followed by the 1260 Infinity II Flexible with the standard configuration.

To demonstrate the retention time precision for these fast chromatographic runs (Table 11), three representative peaks were chosen. Even under these demanding conditions, the retention time precision for both pumps and their corresponding configuration showed excellent values, with <0.09 %RSD.

In an additional experiment, the flow rate was increased from 2 mL/min to 2.6 mL/min, and the resulting system pressure was approximately 750 bar. For this experiment, only the 1260 Infinity II Flexible Pump in standard configuration was used, given that this pump has an operating pressure range of up to 800 bar, whereas the 1260 Infinity II Binary Pump is limited to 600 bar.

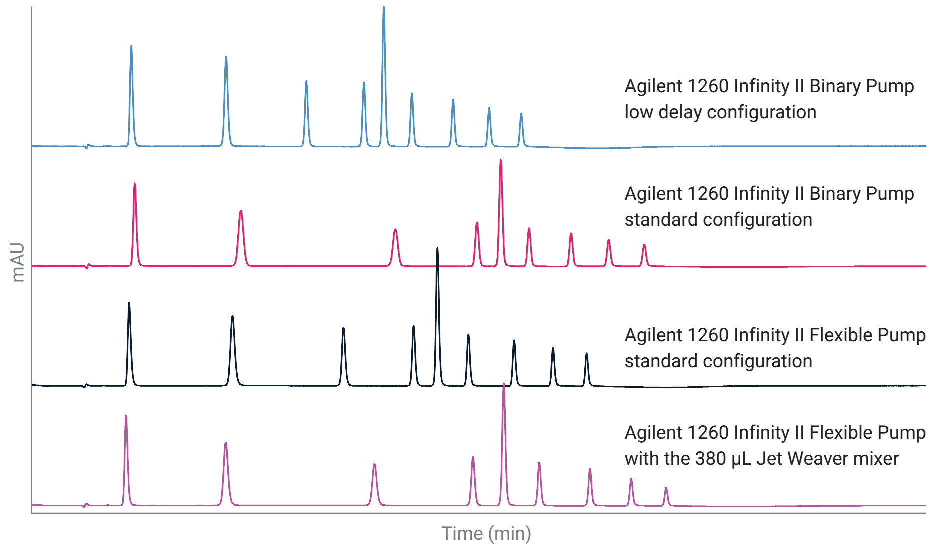

Figure 6. Analysis of the RRLC checkout sample using a high flow rate.

Table 11. Retention time precision for the analysis of the RRLC checkout sample. Three representative peaks were chosen. Eight consecutive runs were used for the calculation.

Agilent 1260 Infinity II Flexible Pump—standard configuration			
	Peak 3	Peak 6	Peak 8
Average	0.697	0.977	1.166
Standard deviation	0.0004	0.0005	0.0005
RSD	0.051	0.047	0.040
Agilent 1260 Infinity II Flexible Pump with 380 µL Jet Weaver mixer			
Average	0.766	1.135	1.341
Standard deviation	0.0005	0.0004	0.0000
RSD	0.060	0.031	0.003
Agilent 1260 Infinity II Binary Pump—standard configuration			
Average	0.813	1.112	1.290
Standard deviation	0.0005	0.0005	0.0006
RSD	0.066	0.048	0.050
Agilent 1260 Infinity II Binary Pump low delay configuration			
Average	0.614	0.850	1.023
Standard deviation	0.0005	0.0006	0.0005
RSD	0.084	0.074	0.047

By increasing the flow rate to 2.6 mL/min, the gradient run time was reduced from 1 to 0.77 minutes (Figure 7). All peaks eluted below one minute, with an outstanding precision of retention times, below the specification of 0.15 %RSD. The last compound eluted earlier (0.967 minutes) with the 1260 Infinity II Flexible Pump than with the 1260 Infinity II Binary Pump (1.095 minutes) with a flow rate of 2 mL/min in low delay configuration.

Conclusion

The Agilent 1260 Infinity II Flexible Pump provided excellent performance regarding retention time precision for various challenging LC applications. The applied step gradients showed excellent composition accuracy and mixing noise for the 1260 Infinity II Flexible Pump and the Agilent 1260 Infinity II Binary Pump. Real-time flow rates were measured and monitored for both pumps, showing that the flow accuracy was always below 0.3 %.

The system delay volume of the 1260 Infinity II Flexible Pump is approximately 310 µL, and 460 µL with the 380 µL Jet Weaver mixer. For the 1260 Infinity II Binary Pump, the system delay volume adds up to 610 µL.

This Technical Overview shows that both pumps demonstrated excellent performance. In addition, the 1260 Infinity II Flexible Pump showed excellent results for demanding applications where a binary pump is normally advantageous.

Compound	Average RT (min)	RSD RT (%)
1. Acetanilide	0.171	0.103
2. Acetophenone	0.351	0.071
3. Propiophenone	0.546	0.037
4. Butyrophenone	0.668	0.021
5. Valerophenone	0.710	0.019
6. Hexanophenone	0.763	0.025
7. Heptanophenone	0.842	0.025
8. Octanophenone	0.909	0.024
9. Benzophenone	0.967	0.024

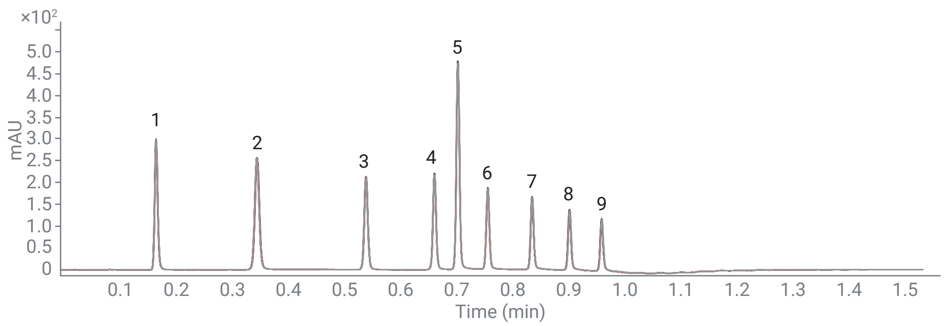

Figure 7. Overlay of eight chromatograms applying a flow rate of 2.6 mL/min at 750 bar backpressure.