Performance characteristics of the Agilent 1290 Infinity Binary Pump

More resolution and speed for conventional, superficially porous and sub-2-micron column packing material

Technical Note

Introduction

The Agilent 1290 Infinity Binary Pump uses new technology to overcome the challenges of pumping LC solvents at ultrahigh pressure and high flow rates. This includes heavy duty drive motors on the pistons; new material for the pistons themselves to withstand the workload and to actively transfer heat from the seals; micro fluidic heat exchangers; and the Jet Weaver, a micro fluidic mixing device. The pump can deliver flow in the range of 0.05 – 5 mL/min at pressures up to 1200 bar.

The Agilent 1290 Infinity Binary Pump contains two identical high pressure pumps (1200 bar) driven by two independent high performance motors each; a two-channel high efficiency solvent degasser and 2 x 2-channel inlet solvent selection valve, automatic purge valve and low-volume mixing device, the Jet Weaver, integrated into a single housing.

One of the main performance criteria of any LC system is precision of retention times, which is influenced primarily by the pumping device. It is important that the desired flow rate is delivered precisely and that for gradient operation the mobile phases are mixed reliably and accurately over the complete gradient range. Because of method transferability and predictability, it is also important that the flow rate and solvent composition in the blending (binary) operation mode are generated accurately. In this Technical Note, the precision of retention times for gradient and isocratic applications is evaluated. Further, based on tracer experiments, it is demonstrated that linear and stepwise gradients are delivered with excellent accuracy and precision.

The new design

The new pump design offers:

- "Infinite" power range (up to 2 mL/min at 1200 bar and 5 mL/min at 800 bar) enables unprecedented-speed and resolution, as well as compatibility with methods developed on other platforms
- Lowest delay volumes down to <10 µL enable ultrafast gradients for LC/MS and LC/UV applications

Innovative Jet Weaver mixer, based on multilayer microfluidics technology, combines highest mixing efficiency with lowest delay volumes to virtually eliminate detector noise

- Dual-core microprocessor-controlled active damping compensates for solvent properties and provides real-time flow optimization to ensure negligible noise and highest precision
- Silicon carbide pistons with superior thermal behavior provide higher seal lifetime and instrument uptime
- Integrated, high efficiency degassing offers fast changeover of solvents for purging and priming the pump

In Figure 1 the pump design is shown schematically. The degasser is now integrated into the pump housing. 2 x 2 solvents can be attached and out of these the binary gradient can be selected.

Each pump channel is a dual piston in-series design using novel firmware control algorithms and innovative piston material, silicon carbide, which efficiently removes frictional heat from the piston seal. The connection between the primary piston to the secondary piston has an integrated heat exchanger to remove the heat generated during solvent compression. Each pump channel has one passive inlet valve and one

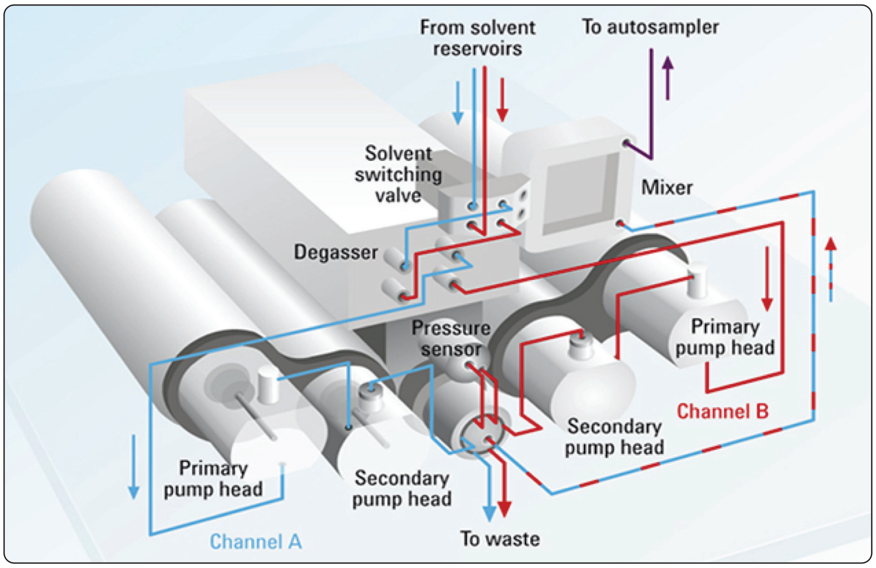


Figure 1
Design of 1290 pump.

passive outlet valve on the primary head. Each piston is independently and precisely driven by a motor with 65000 steps per resolution providing volume displacement resolution as good as 300 pL/step.

The movement of the pistons is under intelligent control with a feedback loop to ensure that active damping results in a ripple-free flow. The piston drive tunes itself for the compressibility characteristics of the solvent and the hydraulic characteristics of the system to maintain the ripple-free state. This in conjunction with the smooth-motion control, which reduces pressure pulsation caused by the movement of the piston, combines with the efficient low-volume mixer to ensure that pump noise on UV traces is the lowest possible. A dedicated microprocessor in the pump takes care of the smooth-motion control and optimization of the pistons' motion for real-time optimization based on the static and dynamic parameters. In addition to superior chromatographic performance these features make the pump very quiet in operation.

For running salt or buffer-containing eluents, the active seal wash option can be used to extend the lifetime of the pump seals.

The solvent selection valve allows the choice of one of two solvents per channel. Binary gradients are created by high-pressure mixing of solvents from channel A and channel B. The mixing point is located within the purge valve thus further reducing the system gradient delay volume. The purge valve allows software controlled flow switching to waste for purging the pump channels.

The mixer design shown in Figure 2 combines highest mixing efficiency with lowest delay volume. Nominal volume mixers of V 35:< 40 µL and V100: < 75 µL (for TFA applications) are possible, just by rotating the mixer assembly. The mixing device, known as the Jet Weaver, employs a multilayer micro fluidic design to ensure optimum suppression of residual composition disturbances. The Jet Weaver is currently available as two nominal volumes: V 35:< 40 µL for normal UV detection applications and V100: < 75 µL for demanding situations such as the use of TFA in UV detection. For MS detection it might be possible to work without the Jet Weaver only making use of natural mixing within the system flow path. Typical applications might be high throughput methods with fast gradients, on high resolution 2.1-mm columns with moderate baseline noise and precision demands. However, the performance specification is only to be considered valid for a system with a Jet Weaver installed.

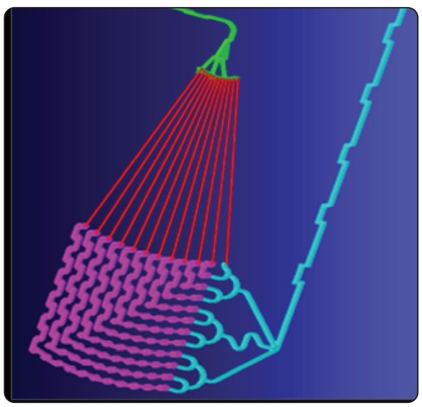


Figure 2
Design of Jet Weaver (mixer).

In the following experiments, the performance of this new design is demonstrated. The pump performance was evaluated using the following:

- Step gradients and linear gradients to evaluate delay volume, noise and ripple, accuracy and precision
- Influence of Jet Weaver mixer
- Retention time (RT) precision at standard conditions for conventional gradient profiles
- RT precision at ultrafast conditions for gradient profiles
- RT precision for fast isocratic runs
- Comparison of acetonitrile and methanol as mobile phase

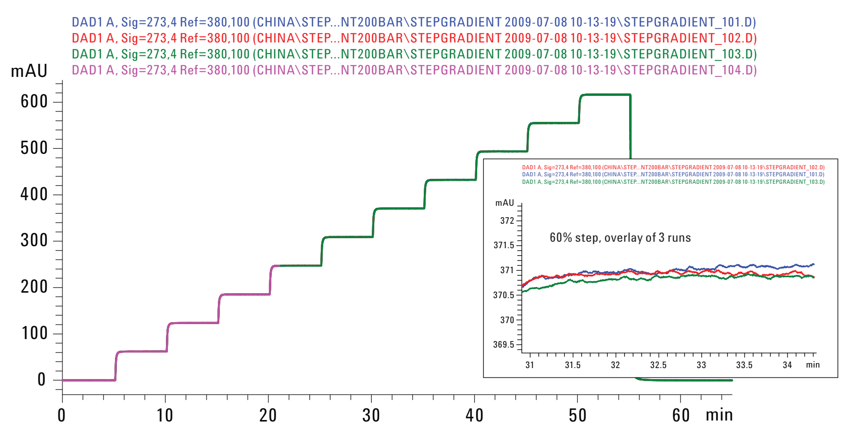
Equipment and material

The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Agilent 1290 Infinity Binary Pump with vacuum degasser
- Agilent 1290 Infinity high performance pump
- Agilent 1290 Infinity TCC
- Agilent 1290 Infinity DAD SL for 160-Hz operation
- Agilent ZORBAX SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particles

Step gradients and linear gradients to evaluate delay volume, noise and ripple, accuracy and precision

To evaluate pump performance tracer experiments are frequently used to verify the system ripple at different gradient mixtures. The delay volume, and the accuracy and precision of gradients are also evaluated using step gradients. Figure 3 shows a step gradient from 0 to 100% in 10% steps using the Agilent 1290 Infinity LC. As a tracer compound, caffeine was selected. Acetone is not ideal for testing step gradient performance because acetone is too easily removed in the degasser at low flow rates. For the 1290 Infinity step gradient performance testing we recommend the use of non-volatile compounds.



Chromatographic conditions:

Column substitute:	Restriction capillaries providing a backpressure of 166 bar 1 mL/min for the solvent used
Mobile phases:	A = water +20% isopropanol, B = water +20% isopropanol +10 mg/l caffeine
Flow rate:	1 mL/min
Step gradients:	0% B to 100% B in 10 % steps, each step held for 5 min
Restrictor temperature:	36 °C
Detection:	Signal 273 nm/4 nm bandwidth, reference 380/100 nm, Slit 4 nm, 20 Hz

Figure 3
Overlay of 3 step gradients.

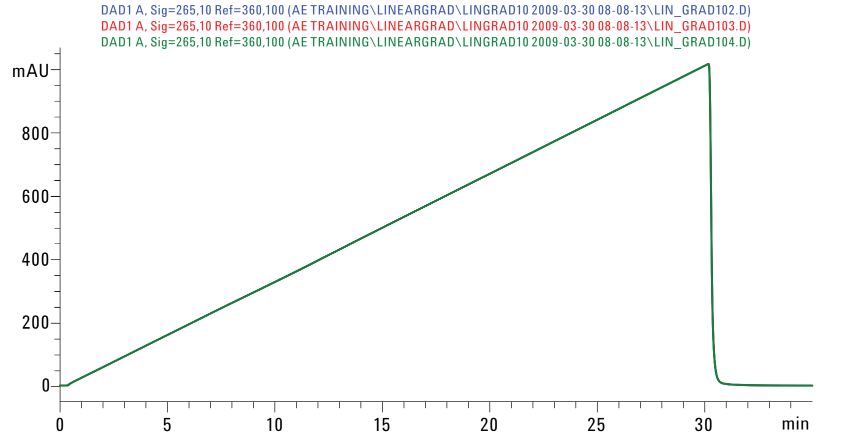
The performance results are:

- Composition Accuracy = ±0.35% or better
- Precision from run to run = <0.1% relative standard deviation (RSD)
- Mixing noise = 0.023% to 0.017% B for critical steps like 10, 50 and 90% step related to 100% step
- System delay volume = <140 µL

In Figure 4, the overlay of three consecutive linear gradients are shown. Precision is also excellent here.

Influence of 35-µL Jet Weaver mixer

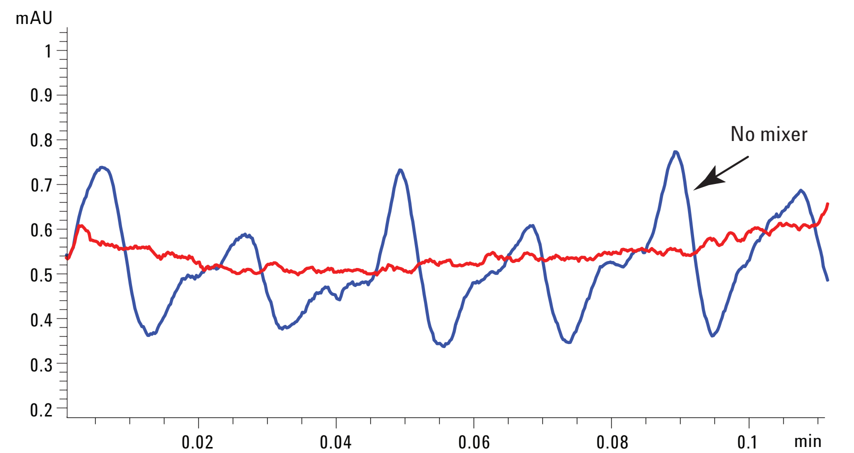
Using UV detection it is advisable to use the V 35:< 40 µL mixer. If this is not sufficient, the V100: < 75 µL mixer should be used, especially if TFA is used as modifier. In Figure 5, the example shows that the 35-µL mixer can mix the mobile phases very efficiently.



Chromatographic conditions:

Column: Restriction capillaries
Mobile phases: A = water, B = water +0.5% acetone
Flow rate: 1 mL/min
Linear gradients: 0% B to 100% B in 30 min
Temperature: 36 °C
Detection: Signal 265/20 nm, reference 360/100 nm, Slit 4 nm, 20 Hz

Figure 4
Overlay of 3 linear gradients.



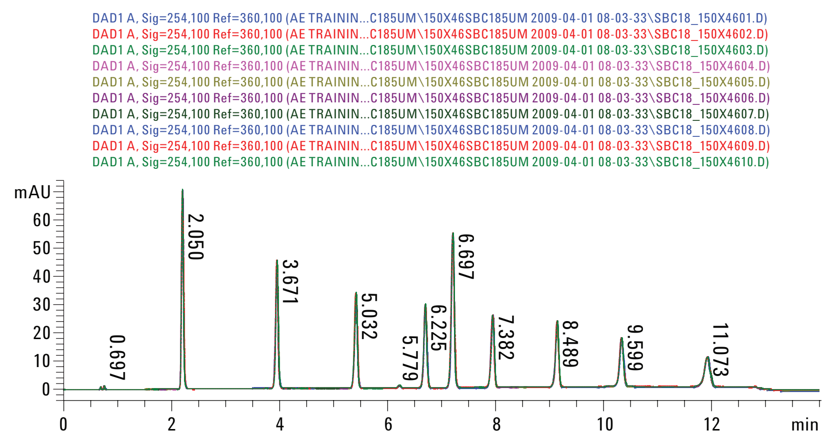
Column: Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm, 1.8 µm,
Sample: Set of 9 compounds, 100 ng/µL each, dissolved in water/ACN (65/35)
1. acetanilide, 2. acetophenone, 3. propiophenone, 4. butyrophenone (200 ng/mL), 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone, 9. octanophenone (p/n 5188-6529)
Mobile phase: water +0.1% TFA /acetonitrile +0.08% TFA with mixer and without mixer
Flow rate: 1 mL/min
Gradient ultrafast: 35% to 95% B in 0.5 min
Oven temperature: 60 °C
DAD: 254/5 nm, REF 400/80 nm, 80 Hz
Injection volume: 0.5 µL, exterior needle wash 6 s

Figure 5
Comparison with and without mixer at 35/65% ACN+0.08%TFA/Water+0.1%TFA.

RT precision at standard conditions for conventional gradient profiles

Retention time precision was tested with gradient and isocratic conditions, using standard bore and narrow bore columns. The relative standard deviation for retention times for conventional chromatography was evaluated using a 150 mm × 4.6 mm standard bore column. A "phenone mix" was analyzed using gradient conditions from 30% to 75% in 8 min and a flow rate of 1.5 mL/min was applied. Figure 6 shows 10 overlaid runs, demonstrating the retention time precision obtained for this application. The run time was 13 min. The evaluation of the retention time precision was found to be less than 0.04 % RSD.

In Table 1 the precision data for conventional runs on 4.6-mm id columns are combined.



Chromatographic conditions:

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
Column:	Agilent ZORBAX SB C18, 150 mm × 4.6 mm, 5 µm
Flow rate:	1.5 mL/min providing a backpressure of 159 bar
Mobile phase:	water/acetonitrile with mixer
Gradient conventional:	30% to 75% B in 8 min
Oven temperature:	30 °C
DAD:	254/10 nm, REF 360/100 nm, 20 Hz
Injection volume:	1 µL, exterior needle wash 6 s

Figure 6
Overlay of 10 chromatograms acquired on 4.6 mm id column.

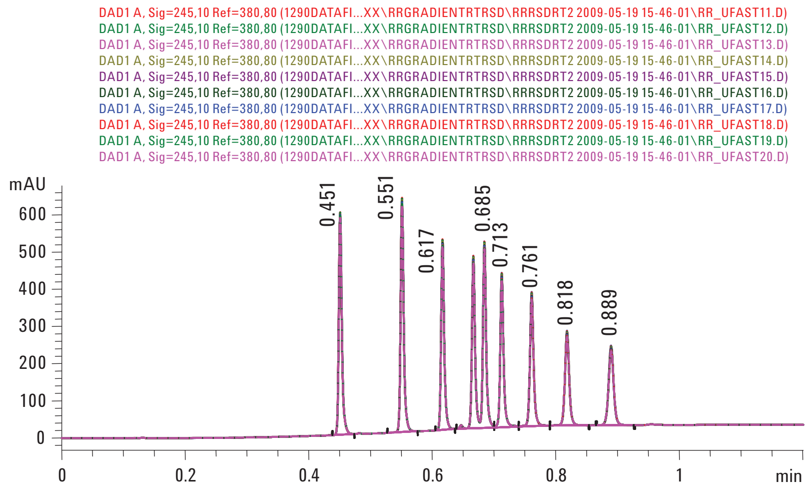
Peak	RSD RT (%)
1	0.026
2	0.034
3	0.031
4	0.028
5	0.028
6	0.026
7	0.024
8	0.027
9	**0.035**

Table 1
Precision of retention times for conventional runs on 150 mm × 4.6 mm column.

RT precision at ultrafast conditions for gradient profiles

In Figure 7, an example of an ultrafast separation with a gradient time of 0.5 min is presented. The flow was set to 1.2 mL/min. All peaks elute within 1 min. Peak width at half height for the first peak is as narrow as 0.334 sec and for the last peak the peak width is only 0.543 sec. Even for this demanding application, the precision of retention times was <0.008% RSD except for the first peak.

In Table 2, the precision data for ultrafast runs on 2.1-mm id columns are combined.



Column:	Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm, 1.8 µm,
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:	water/acetonitrile with mixer
Gradient:	At 0 min 5% ACN, at 0.5 min 95% ACN
Flow rate:	1.2 mL/min
Oven temperature:	40 °C
DAD:	245/10 nm, REF 360/80 nm, 80 Hz
Injection volume:	0.5 µL exterior needle wash 6 s

Figure 7
Overlay of 10 ultrafast runs with a gradient time of 0.5 min at 715 bar backpressure.

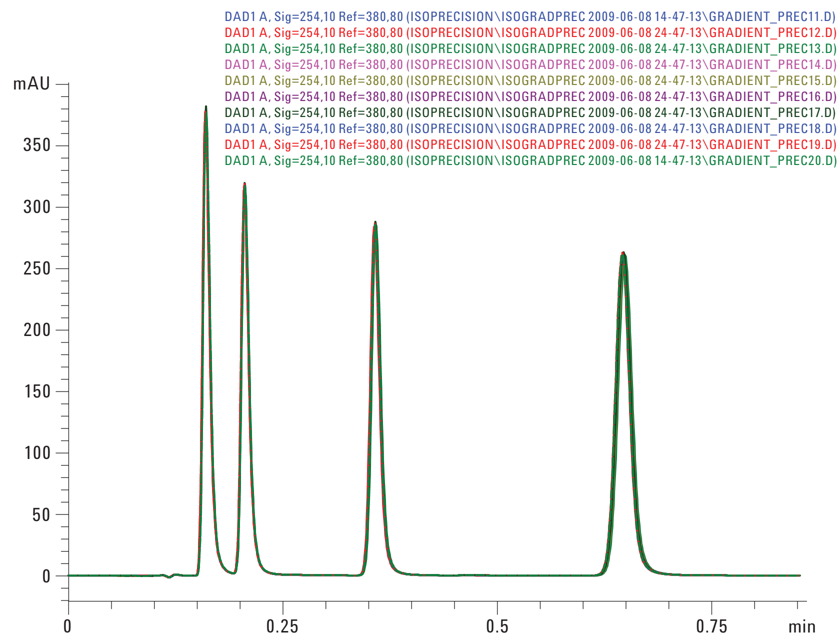
Peak	RSD RT over 10 consecutive runs
1	0.016
2	0.00769
3	0.00517
4	0.00575
5	0.00577
6	0.00538
7	0.00585
8	0.00571
9	0.00797

Table 2
Precision of retention times for ultra fast gradient runs.

RT precision for ultrafast isocratic runs at 670 bar backpressure

In Figure 8, an example for isocratic conditions is shown. A narrow bore column was chosen and the flow rate was set to 1 mL/min. The mobile phases were blended in the pump, delivered by the two pump channels and mixed in the 35-µL mixer. The four peaks elute within 45 sec. The standard deviation is typically <70 msec for these peaks.

In Table 3, the results are combined.



Column:	Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm, 1.8 µm, 1200 bar
Sample:	Agilent isocratic test sample with dimethylphthalate, diethylphthalate, biphenyl, o-terphenyl,
Mobile phase:	water/acetonitrile, dynamically blended with mixer = 20/80
Flow rate:	1 mL/min
Oven temperature:	50 °C
DAD:	254/10 nm, REF 360/80 nm, 80 Hz
Injection volume:	0.5 µL, exterior needle wash 6 s

Figure 8
Precision of retention times on 2.1-mm column, isocratic elution, overlay of 10 runs, SD of RT less than 75 msec. 35 µL Jet Weaver used for mixing. Backpressure was 670 bar.

Peak	MeanRT (sec)	SD (msec)	RSD (%)
1	9.6	19	0.194
2	12.3	18	0.147
3	21.42	33	0.156
4	38.76	68	0.176

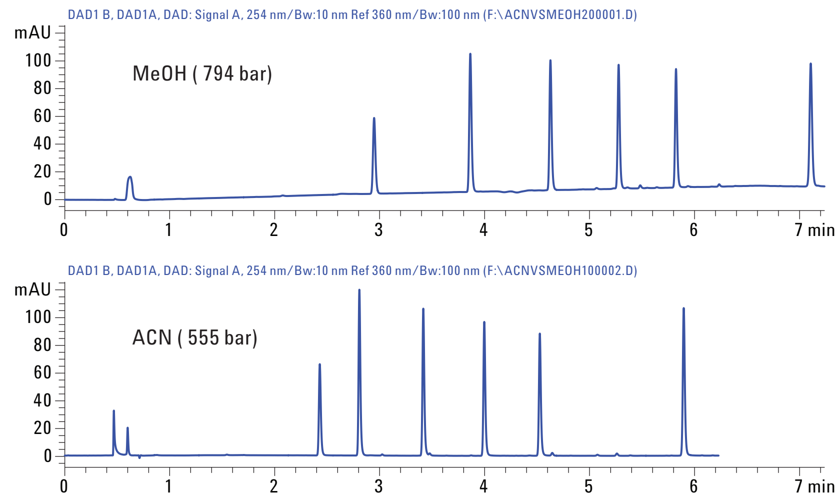
Table 3
Precision of retention times for fast isocratic runs.

Comparison of methanol and acetonitrile as mobile phase

Because of the recent economic situation, there has been a shortage of acetonitrile. Therefore, it is important that the LC system can operate with methanol instead of acetonitrile. Methanol and its aqueous mixtures have a higher viscosity than acetonitrile, resulting in higher back pressures under the same chromatographic conditions. Methanol can be used with the Agilent 1290 Infinity LC system without exceeding the instrument pressure limit. Figure 9 illustrates the influence of methanol on backpressure and peak elution.

Conclusion

The data presented in this note show that the precision of retention time of the Agilent 1290 Infinity LC system is excellent for a wide range of LC applications using either narrow bore or standard bore columns. The retention time precision is typically less than 0.075% relative standard deviation for conventional flow rates and ultrafast applications with run times less than 1 min. Accuracy and precision of linear and step gradients are excellent. Pump ripple is typically less than 0.025%. The total system delay volume is 105 µL without a mixer and 140 µl with a mixer.



Sample:	Gradient Evaluation mix, No.48271, sample contains: uracil, phenol, methylparaben, ethylparaben, propylparaben, butylparaben, heptylparaben, supplier is Sigma Aldrich
Column:	Agilent ZORBAX RRHD SB C18, 2.1 mm × 100 mm, 1.8 µm,
Mobile phases:	water/acetonitrile or methanol (B)
Gradient:	at 0 min 5% B, at 6 min 80% B, at 6.5 min 80% B, at 6.6 min 5% B, at 8 min 5% B
Flow rate:	0.5 mL/min
Column temperature:	40 °C
Detection:	254 nm, 20 Hz
Injection volume:	1 µL

Figure 9
Comparison of acetonitrile and methanol as mobile phases.

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