Performance characteristics of the Agilent 1290 Infinity Binary Pump

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

Technical Overview

Introduction

The Agilent 1290 Infinity Binary Pump uses 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 to withstand the workload and 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 1290 Infinity Binary Pump contains; two identical high pressure pumps (1,200 bar), each driven by an independent high performance motor; a two-channel high efficiency solvent degasser with a 2 × 2-channel inlet solvent selection valve; an automatic purge valve; and the Jet Weaver, a low-volume mixing device. Everything is 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. This Technical Note evaluates the precision of retention times for gradient and isocratic applications. It also demonstrates that, based on tracer experiments, linear and stepwise gradients are delivered with excellent accuracy and precision.

Pump design

- The power range (up to 2 mL/min at 1,200 bar and 5 mL/min at 800 bar) that 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.
- The 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.
- Integrated, high efficiency degassing offers fast changeover of solvents for purging and priming the pump.

Figure 1 shows the pump design schematically. The degasser is now integrated into the pump housing. Binary gradients can be selected from attached 2 × 2 solvents.

Each pump channel is a dual piston in series design using novel firmware control algorithms to ensure lowest pumping ripple. 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 passive outlet valve on the primary head. Each piston is independently and precisely driven by a motor with 65,000 steps per resolution providing volume displacement resolution as good as 300 pL/step.

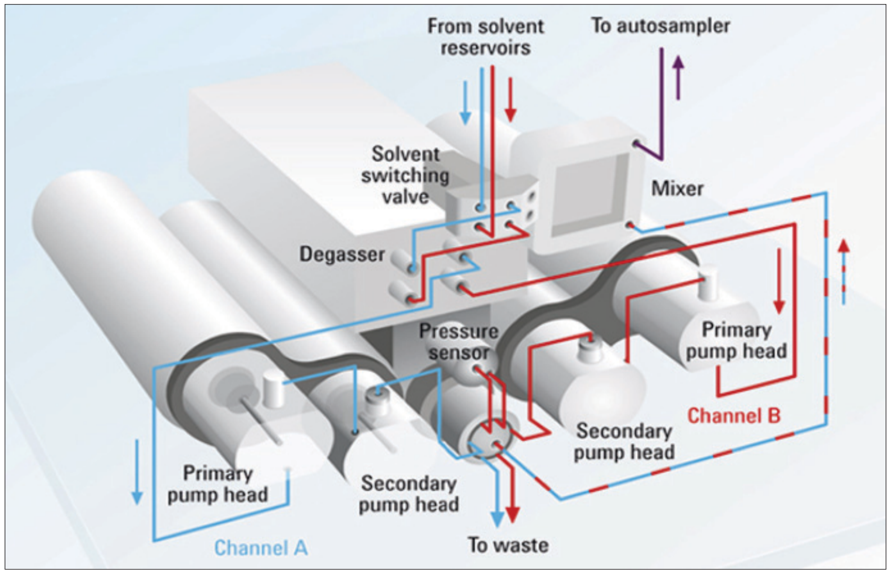


Figure 1
Design of 1290 Infinity Binary Pump.

The movement of the pistons is controlled 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 ensures 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, by rotating the mixer assembly. The mixing device, the Jet Weaver, employs a multilayer micro fluidic design to ensure optimum suppression of residual composition disturbances. The Jet Weaver is 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 using only 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 considered valid for a system with a Jet Weaver installed.

The following experiments demonstrate the performance of this design. 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

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 1290 Infinity LC System. As a tracer compound, caffeine was selected. Acetone is not ideal for testing step gradient performance because it is too easily removed in the degasser at low flow rates. For the 1290 Infinity LC System step gradient performance testing, we recommend using nonvolatile compounds.

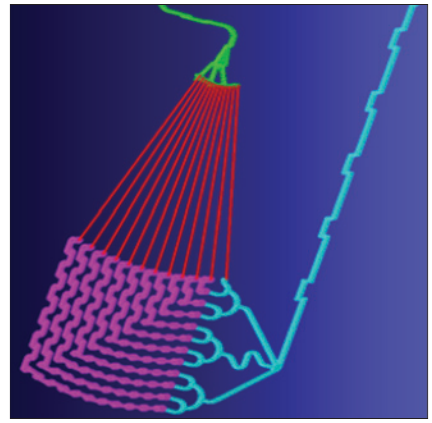
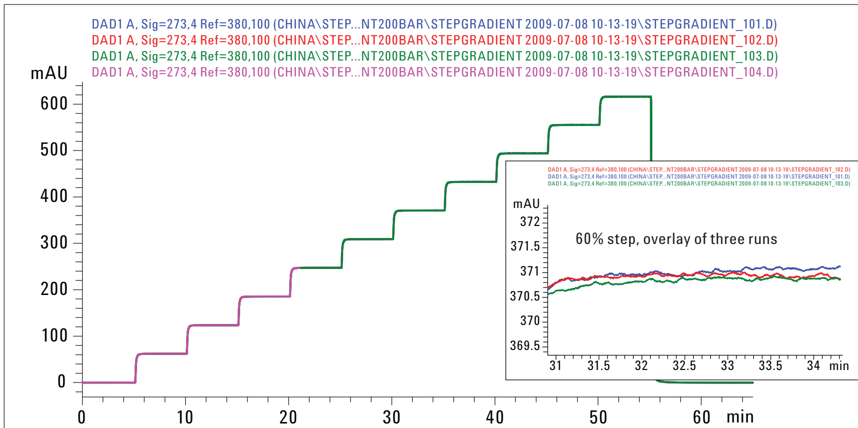


Figure 2
Design of a Jet Weaver (mixer).



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 minutes
Restrictor temperature:	36 °C
Detection:	Signal 273 nm/4 nm bandwidth, reference 380/100 nm, Slit 4 nm, 20 Hz

Figure 3
Overlay of three step gradients.

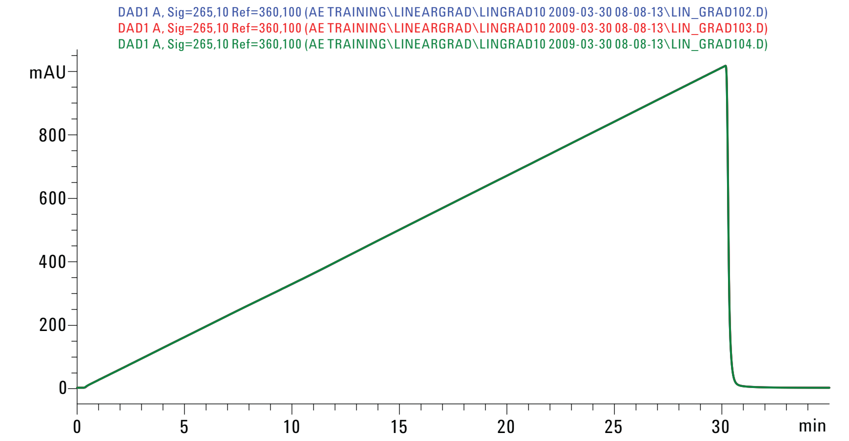
The performance results are:

- 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

Figure 4 shows the overlay of three consecutive linear gradients. Precision is also excellent here.

Influence of a 35-µL Jet Weaver mixer

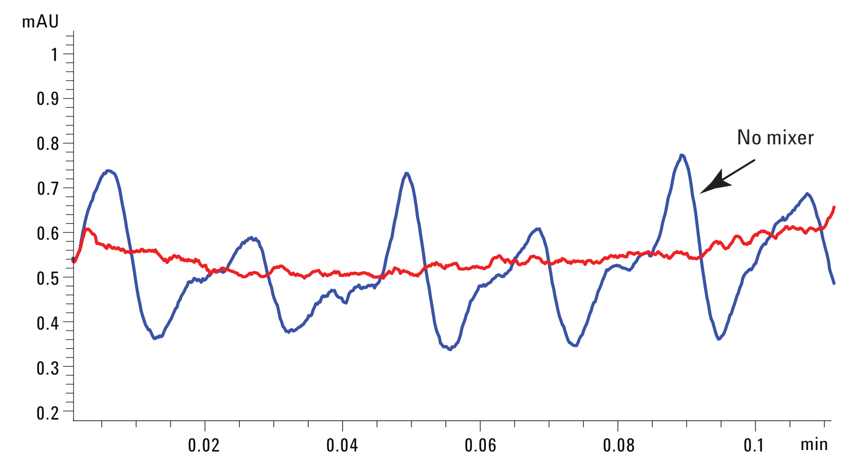
It is advisable to use the V 35:< 40-µL mixer when using UV detection. If this is not sufficient, use the V100:< 75 µL mixer, especially if TFA is used as modifier. Figure 5 shows that the 35-µL mixer can mix the mobile phases very efficiently.



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 minutes
Temperature:	36 °C
Detection:	Signal 265/20 nm, reference 360/100 nm, Slit 4 nm, 20 Hz

Figure 4
Overlay of three linear gradients.



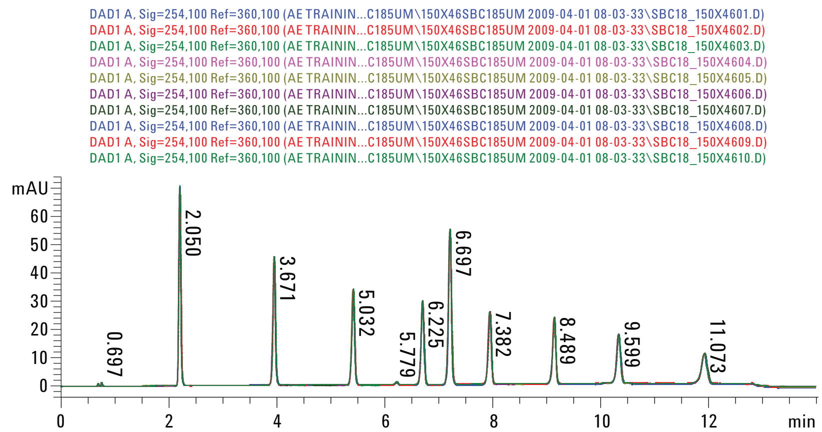
Column:	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm,
Sample:	Set of nine compounds, 100 ng/µL each, dissolved in water/ACN (65/35) 1. acetanilide, 2. acetophenone, 3. propiophenone, 4. butyrophenone (200 ng/mL), 5. benzophenone, 6. valerophenone, 7. hexanophenone, 8. heptanophenone, 9. octanophenone (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 minutes
Oven temperature:	60 °C
DAD:	254/5 nm, REF 400/80 nm, 80 Hz
Injection volume:	0.5 µL, exterior needle wash 6 seconds

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 4.6 × 150 mm standard bore column. A phenone mix was analyzed using gradient conditions from 30% to 75% in 8 minutes 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 minutes. The evaluation of the retention time precision was found to be less than 0.04 % RSD.

Table 1 combines the precision data for conventional runs on 4.6-mm id columns.



Chromatographic conditions

Sample:	Set of nine 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, 4.6 × 150 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 minutes
Oven temperature:	30 °C
DAD:	254/10 nm, REF 360/100 nm, 20 Hz
Injection volume:	1 µL, exterior needle wash 6 seconds

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

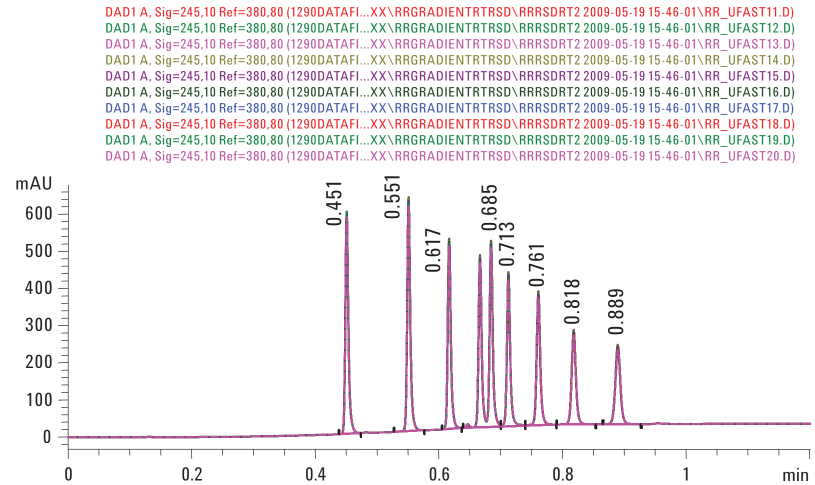
Peak	RSD RT (%)
1	0.026
2	0.034
3	0.031
4	0.028
5	0.028
6	0.026
7	0.024
8	0.027
9	0.035

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

RT precision at ultrafast conditions for gradient profiles

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

Table 2 combines the precision data for ultra-fast runs on 2.1-mm id columns.



Column:	Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm,
Sample:	Set of nine 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 minutes 5% ACN, at 0.5 min 95% ACN
Flow rate:	1.2 mL/minutes
Oven temperature:	40 °C
DAD:	245/10 nm, REF 360/80 nm, 80 Hz
Injection volume:	0.5 µL exterior needle wash 6 seconds

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

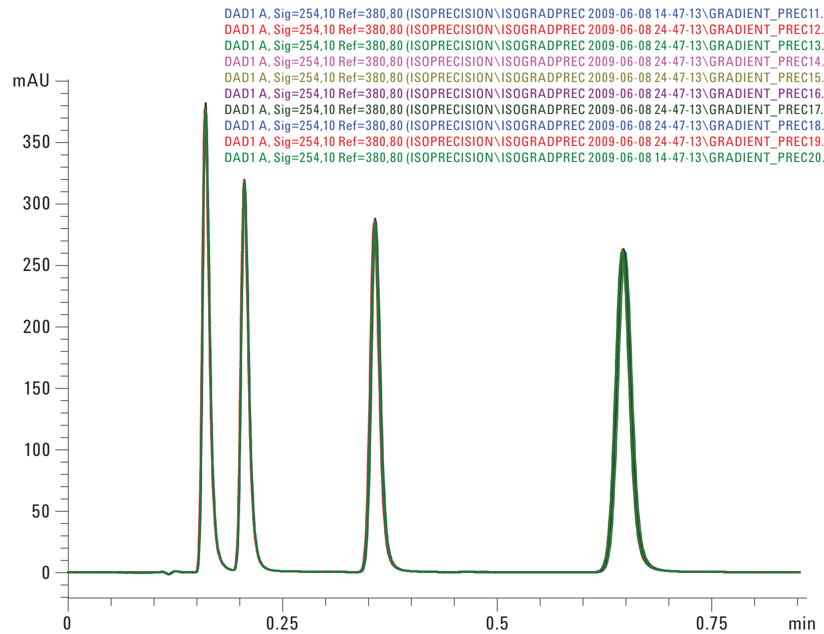
Peak	RSD RT over 10 consecutive runs
1	0.016
2	0.00769
3	0.00517
4	0.00575
5	0.00577
6	0.00538
7	0.00585
8	0.00571
9	0.00797

Table 2
Precision of retention times for ultrafast gradient runs.

RT precision for ultrafast isocratic runs at 670 bar backpressure

Figure 8 shows an example for isocratic conditions. 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 seconds. The standard deviation is typically < 70 msec for these peaks.

Table 3 combines the results.



Column: Agilent ZORBAX RRHD SB C18, 2.1 × 50 mm, 1.8 µm, 1,200 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/minutes
Oven temperature: 50 °C
DAD: 254/10 nm, REF 360/80 nm, 80 Hz
Injection volume: 0.5 µL, exterior needle wash 6 seconds

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

Peak	Mean RT (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

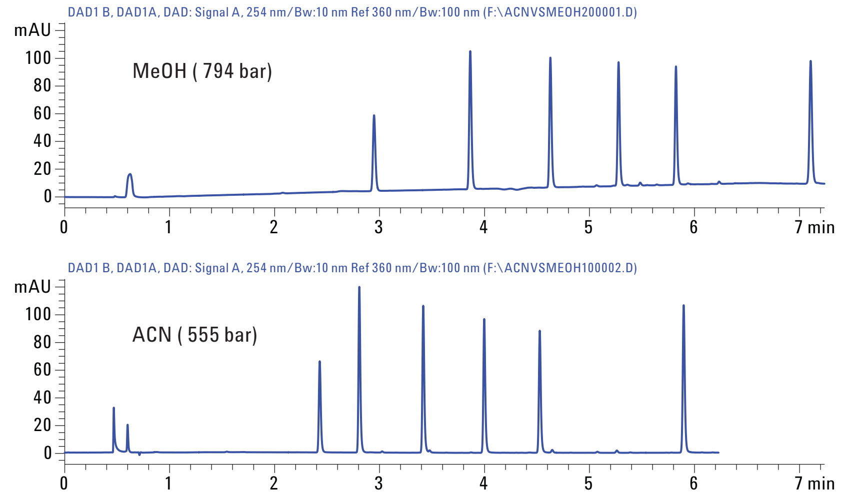
Table 3
Precision of retention times for fast isocratic runs.

Comparison of methanol and acetonitrile as mobile phase

Because of the 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 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 Technical 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 minute. 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 × 100 mm, 1.8 µm,
Mobile phases:	water/acetonitrile or methanol (B)
Gradient:	at 0 minutes 5% B, at 6 minutes 80% B, at 6.5 minutes 80% B, at 6.6 minutes 5% B, at 8 minutes 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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