# Performance Characteristics of the Agilent 1260 Infinity II Bio-Inert LC

## Technical Overview


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### Introduction

The Agilent 1260 Infinity II Bio-inert LC is the next generation of Agilent Bio-inert LC, specially designed for conditions used in biochromatography (high salt concentrations such as 2 M NaCl, up to 8 M urea, and high and low pH solvents such as 0.5 M NaOH or 0.5 M HCl) by working with a completely inert sample flowpath. All capillaries and fittings throughout the multisampler, multicolumn thermostat, and detectors, are metal-free, and biomolecules come in contact only with ceramics or PEEK.

Design elements in the Agilent 1260 Infinity II Bio-inert Multisampler and Agilent 1260 Infinity II Bio-inert Multicolumn Thermostat (MCT) provide the user with the highest flexibility. Based on the proven technology of the Agilent 1260 Infinity Series liquid chromatography platform, the 1260 Infinity II Bio-inert LC has equivalent performance specifications as the standard 1260 Infinity Bio-inert Quaternary LC[1].

The 1260 Infinity II Bio-inert LC is compatible with standard methods, with a maximum pressure of 600 bar.


Agilent Technologies

The 1260 Infinity II Bio-inert LC offers:

- Flow rate range up to 10 mL/min
- Maximum pressure of 600 bar for UHPLC. Higher pressures allow support of either higher flow rates (more speed) or longer columns (more resolution).
- The Agilent 1260 Infinity II Bio-inert Multisampler is based on a proven flow-through design using a ceramic needle. Further elements are the sample hotel for various sample containers that hold up to eight vial or microtiter plates, the robotics and needle handling routine for shortest injection cycle times, and the multiwash function for lowest carryover.
- An advanced column capacity for up to six columns, with individual precolumn solvent heating in a single MCT. The door provides superior usability with flexible flap positions at 90 or 180°, and it can be removed completely for highest accessibility. The MCT facilitates precise column thermostatting for maximum application flexibility. These performances are possible by Peltier cooling and heating with two independent temperature zones from 10 degrees below ambient (minimum 4 °C) to 85 °C. In addition, the MCT can be equipped with a column tag reading option for automatic and trackable column management[2].
- Agilent 1260 Infinity II LC Diode Array Detector (DAD) WR with bio-inert standard flow cell
- Standard-bore UHPLC and conventional applications run on the same system configuration.
- Revolutionary Agilent InfinityLab Quick Connect UHPLC column fittings for dead-volume-free fluidic connections that enable efficient, fast, and convenient column exchange. In addition, easy-to-install precolumn Agilent InfinityLab Quick-Connect heat exchangers are available for precolumn solvent heating (Figure 1).

## Experimental

The 1260 Infinity II Bio-inert LC that was tested comprised:

- Agilent 1260 Infinity II Bio-inert Pump (G5654A)
- Agilent 1260 Infinity II Bio-inert Multisampler (G5668A) with sample cooler (Option #100)
- Agilent 1260 Infinity II Multicolumn Thermostat (G7116A) with bio-inert heat exchanger (Option #019)
- Agilent 1260 Infinity II Diode Array Detector WR (G7115A) with bio-inert flow cell (Option #028)

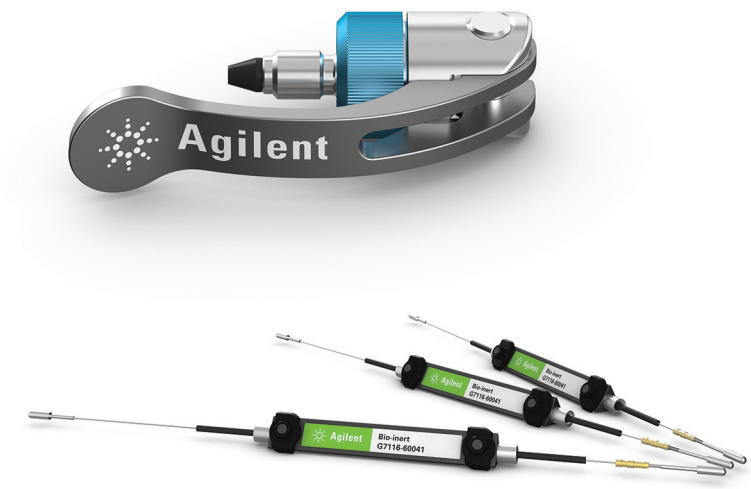
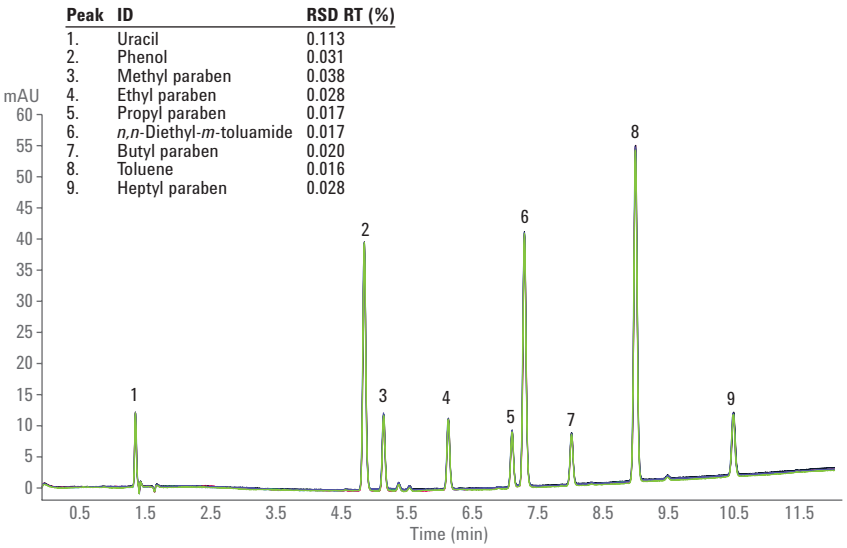



Figure 1. Agilent InfinityLab Quick Connect fittings and Agilent InfinityLab Quick-Connect heat exchangers.

## Pump performance – retention time (RT) precision

Themostimportantparameterinfluencing retention time (RT) precision is pump performance. RT precision was tested with different gradient and isocratic conditions using 4.6 and 2.1 mm id columns. The relative standard deviation (RSD) of RTs for conventional gradient runs was <0.04 %RSD, except for the first peak for n = 7 (Figure 2.)

For fast gradients, with a run time of approximately 1 minute, the RSD for RTs was <0.12 %RSD (Figure 3) for n = 7.




| Peak | ID | RSD RT (%) |
|---|---|---|
| 1. | Uracil | 0.113 |
| 2. | Phenol | 0.031 |
| 3. | Methyl paraben | 0.038 |
| 4. | Ethyl paraben | 0.028 |
| 5. | Propyl paraben | 0.017 |
| 6. | *n,n*-Diethyl-*m*-toluamide | 0.017 |
| 7. | Butyl paraben | 0.020 |
| 8. | Toluene | 0.016 |
| 9. | Heptyl paraben | 0.028 |

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 paraben, ethyl paraben, propyl paraben, butyl paraben, heptyl paraben, uracil)

**Sample preparation**

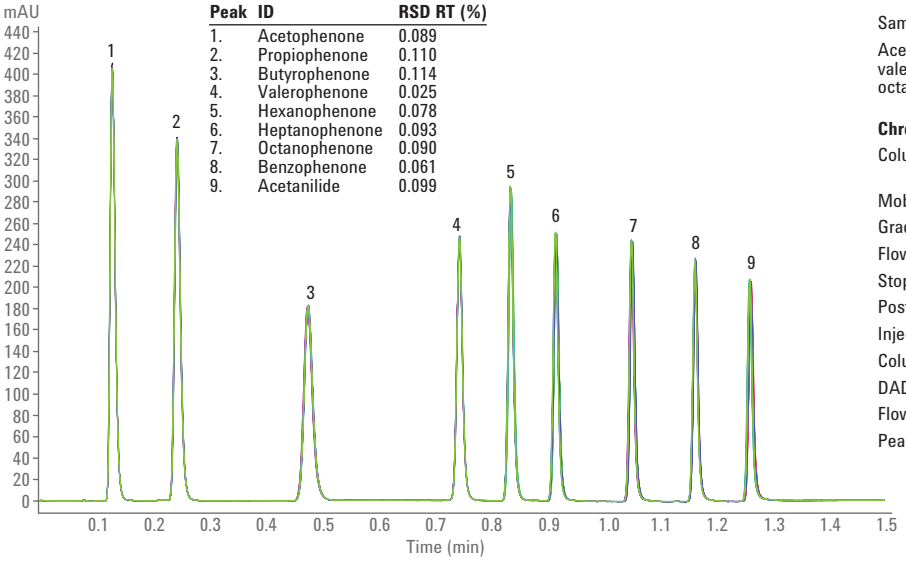
Dilute each sample to 5 mL with water/acetonitrile 1:1

Mix the two diluted samples 1:1

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 5 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Gradient | 0 minutes 20 %B, 10 minutes 95 %B |
| Flow rate | 1 mL/min |
| Stop time | 12 minutes |
| Post time | 5 minutes |
| Injection volume | 5 µL |
| Column temperature | 30 °C |
| DAD | 254/4 nm; Ref 400/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.025 minutes (10 Hz) |

Figure 2. Precision of RTs for conventional gradient runs.




| Peak | ID | RSD RT (%) |
|---|---|---|
| 1. | Acetophenone | 0.089 |
| 2. | Propiophenone | 0.110 |
| 3. | Butyrophenone | 0.114 |
| 4. | Valerophenone | 0.025 |
| 5. | Hexanophenone | 0.078 |
| 6. | Heptanophenone | 0.093 |
| 7. | Octanophenone | 0.090 |
| 8. | Benzophenone | 0.061 |
| 9. | Acetanilide | 0.099 |

Sample: RRLC Checkout sample (p/n 5188-6529)

Acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, benzophenone, acetanilide

**Chromatographic conditions**

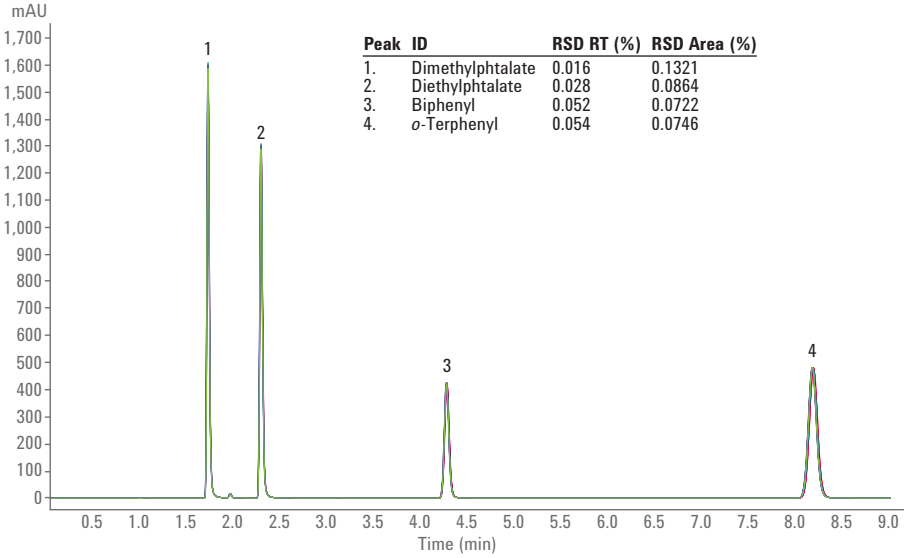
| | |
|---|---|
| Column | Agilent Poroshell 120 EC C18, 2.1 mm × 50 mm, 2.7 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Gradient | 0 minutes 30 %B – 1 minute 95 %B |
| Flow rate | 2 mL/min |
| Stop time | 1.5 minutes |
| Post time | 1 minute |
| Injection volume | 1 µL |
| Column temperature | 50 °C |
| DAD | 245/10 nm; Ref 360/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.00625 minutes (40 Hz) |

Figure 3. Precision of RTs for fast gradient runs.

Figure 4 shows conventional isocratic conditions with an RT precision of <0.06 %RSD for n = 7.

## Comparison of an Agilent 1260 Infinity Bio-inert Quaternary LC and an Agilent 1260 Infinity II Bio-inert LC – step gradient

Tracer experiments are frequently used to verify the solvent mixing ripple at different gradient mixtures to evaluate pump performance. Figure 5 shows a step gradient from 0 to 100 % in 10 % steps with caffeine as the tracer compound. An overlay of step gradients generated with the 1260 Infinity Bio-inert Quaternary LC and the 1260 Infinity II Bio-inert LC is shown. The performance of both systems is highly comparable regarding the mixing properties of the pump.



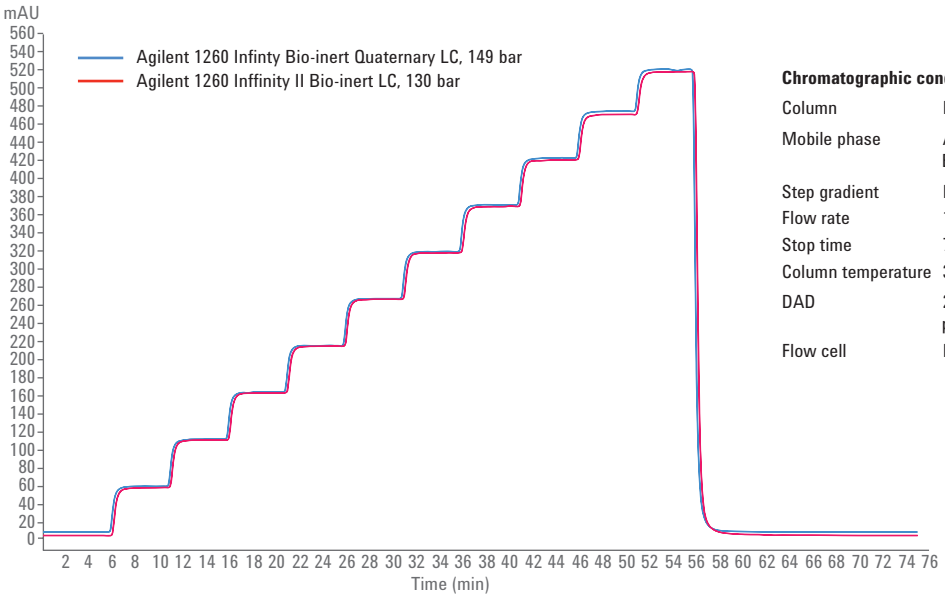

| Peak | ID | RSD RT (%) | RSD Area (%) |
|---|---|---|---|
| 1. | Dimethylphtalate | 0.016 | 0.1321 |
| 2. | Diethylphtalate | 0.028 | 0.0864 |
| 3. | Biphenyl | 0.052 | 0.0722 |
| 4. | *o*-Terphenyl | 0.054 | 0.0746 |

Isocratic sample (p/n 01080-68707)

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent ZORBAX SB C18, 4.6 × 150 mm, 1.8 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Isocratic | 70 %B |
| Flow rate | 1.2 mL/min |
| Stop time | 9 minutes |
| Injection volume | 5 µL, draw speed 200 µL/min |
| Column temperature | 40 °C |
| DAD | 254/40 nm; Ref 380/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.025 minutes, (10 Hz) |

Figure 4. RT precision for conventional isocratic runs.




**Chromatographic conditions**

| | |
|---|---|
| Column | Restriction capillary |
| Mobile phase | A) Water + 20 % isopropanol<br>B) Water + 20 % isopropanol + 10 mg/L caffeine |
| Step gradient | From 0 to 100% B in 10% steps |
| Flow rate | 1 mL/min |
| Stop time | 75 minutes |
| Column temperature | 36 °C |
| DAD | 273/4 nm; Ref 380/100 nm;<br>peak width: <0.013 minutes, (20 Hz) |
| Flow cell | Bio-inert standard flow cell, 10 mm |

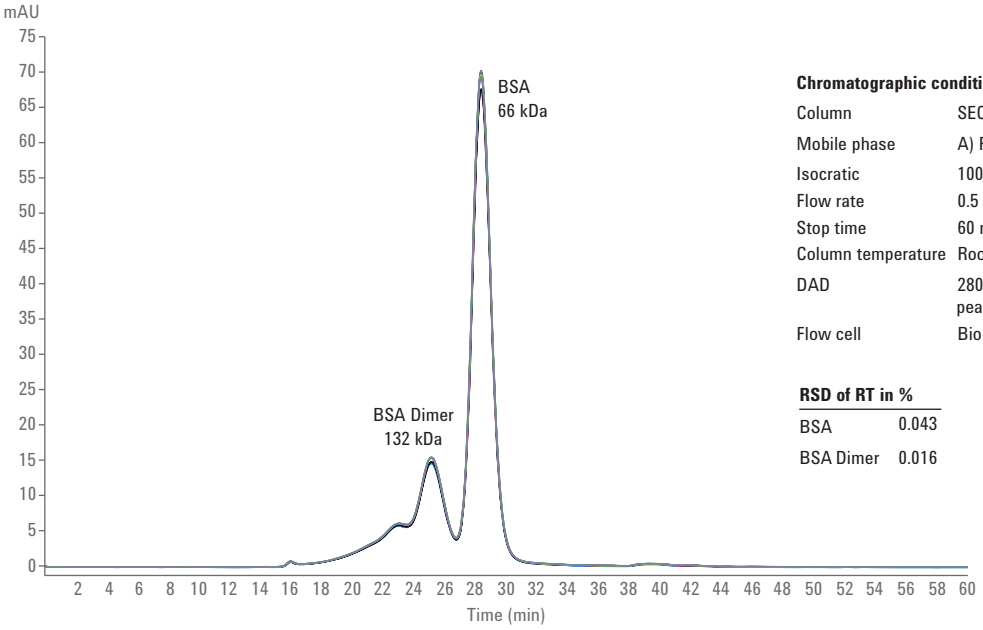
Figure 5. Overlay of step gradients. Agilent 1260 Infinity Bio-inert Quaternary LC and an Agilent 1260 Infinity II Bio-inert LC.

## Low backpressure application – protein separation by size exclusion chromatography (SEC) at 21 bar

Low backpressure applications, commonly used in protein analysis, were tested with the 1260 Infinity II Bio-inert LC to prove pressure stability and precision of RT with the 600 bar system. High precision of RTs was obtained (RSD of <0.045 %, n = 5). Figure 6 shows a separation of bovine serum albumin (BSA) from its dimer using an SEC column with a low backpressure, resulting in a total system pressure of 21 bar.

## Injector performance – area precision

Precise injection is mandatory for good quantitative results in liquid chromatography. The 1260 Infinity II Bio-inert Multisampler can inject precisely over an injection range of 0.5 to 100 µL. Figure 7 shows an example chromatogram for an injection volume of 1 µL. The RSD was <0.37 % for n = 7. The RSD for an injection volume of 0.5 µL was <0.42 % for n = 7.



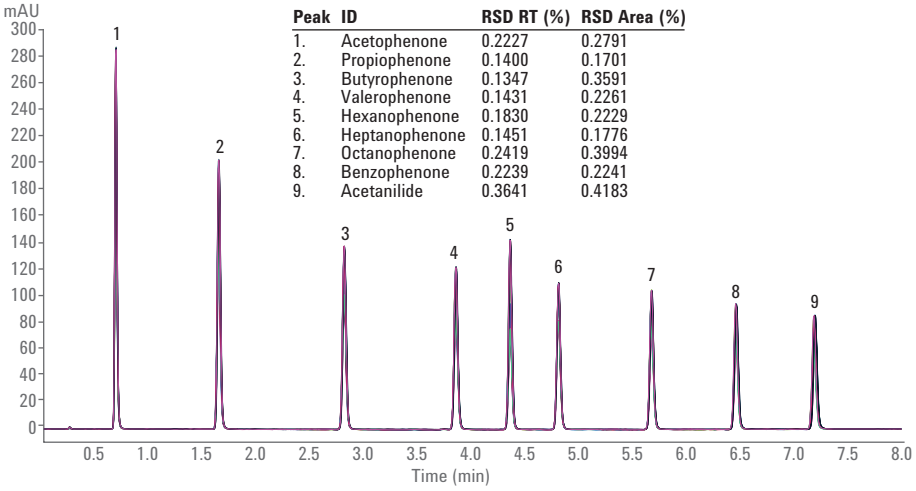

**Chromatographic conditions**

| | |
|---|---|
| Column | SEC, 10 × 300 mm, 13 µm |
| Mobile phase | A) Phosphate buffered saline, pH = 7.2 |
| Isocratic | 100 %A |
| Flow rate | 0.5 mL/min |
| Stop time | 60 minutes |
| Column temperature | Room temperature |
| DAD | 280/4 nm; Ref 360/100 nm; peak width: <0.10 minutes, (2.5 Hz) |
| Flow cell | Bio-inert standard flow cell, 10 mm |

**RSD of RT in %**

| | |
|---|---|
| BSA | 0.043 |
| BSA Dimer | 0.016 |

Figure 6. Low backpressure application: separation of bovine serum albumin (BSA) at 21 bar.




| Peak | ID | RSD RT (%) | RSD Area (%) |
|---|---|---|---|
| 1. | Acetophenone | 0.2227 | 0.2791 |
| 2. | Propiophenone | 0.1400 | 0.1701 |
| 3. | Butyrophenone | 0.1347 | 0.3591 |
| 4. | Valerophenone | 0.1431 | 0.2261 |
| 5. | Hexanophenone | 0.1830 | 0.2229 |
| 6. | Heptanophenone | 0.1451 | 0.1776 |
| 7. | Octanophenone | 0.2419 | 0.3994 |
| 8. | Benzophenone | 0.2239 | 0.2241 |
| 9. | Acetanilide | 0.3641 | 0.4183 |

Sample: RRLC Checkout sample (p/n 5188-6529)

Acetophenone, propiophenone, butyrophenone, valerophenone, hexanophenone, heptanophenone, octanophenone, benzophenone, acetanilide

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent Poroshell 120 EC C18, 3 × 50 mm, 2.7 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Gradient | 0 minutes 20 %B, 8 minutes 80 %B |
| Flow rate | 1.2 mL/min |
| Stop time | 8 minutes |
| Post time | 4 minutes |
| Injection volume | 1 µL |
| Column temperature | 30 °C |
| DAD | 245/10 nm; Ref 400/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.025 minutes (10 Hz) |

Figure 7. Area precision for conventional gradient runs for 1 and 0.5 µL.

Figure 8 shows an example chromatogram for an injection volume of 5 µL. The RSD is <0.14 % for n = 7.

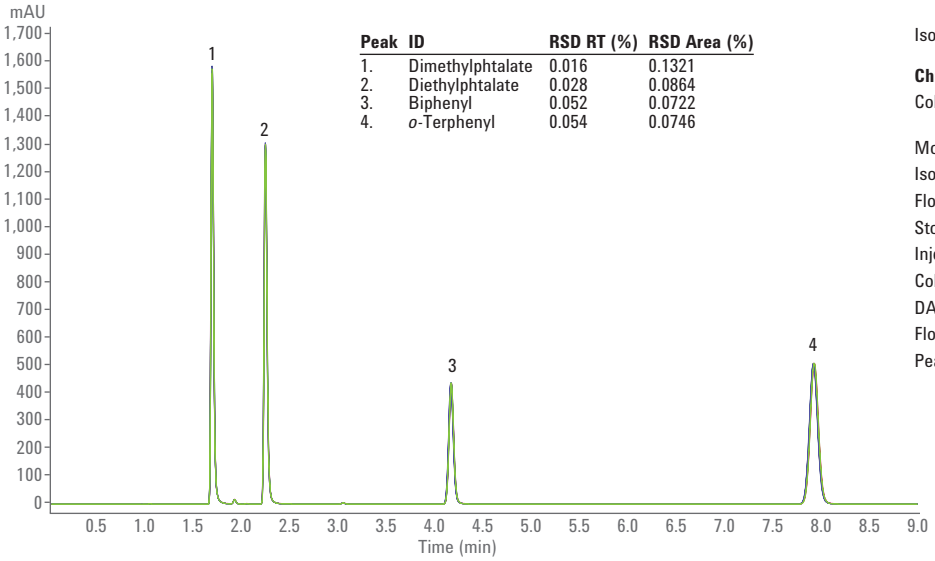
The injector settings are important for optimum precision of areas. If the highest precision is needed, the draw speed of the injector should be set to lower values, especially if large volumes or highly viscous samples are injected. It is important to avoid solvent evaporation out of the sample vials, and decomposition problems using a cooled autosampler.

## Injector performance – carryover

For the injection, the draw speed was set to 20 µL/min, and an exterior needle wash for 10 seconds was used (Figure 9). No carryover was detected for the conditions used. After a 1,000-ng sample injection, unadulterated solvent was injected.

## Recommendations for carryover and cleaning procedures

Flush port wash solvent must always be installed and used. The solvent chosen should be able to dissolve the sample compounds. It is highly recommended to reconnect the capillary connections from time to time to prevent cavities, which can lead to enhanced carryover.



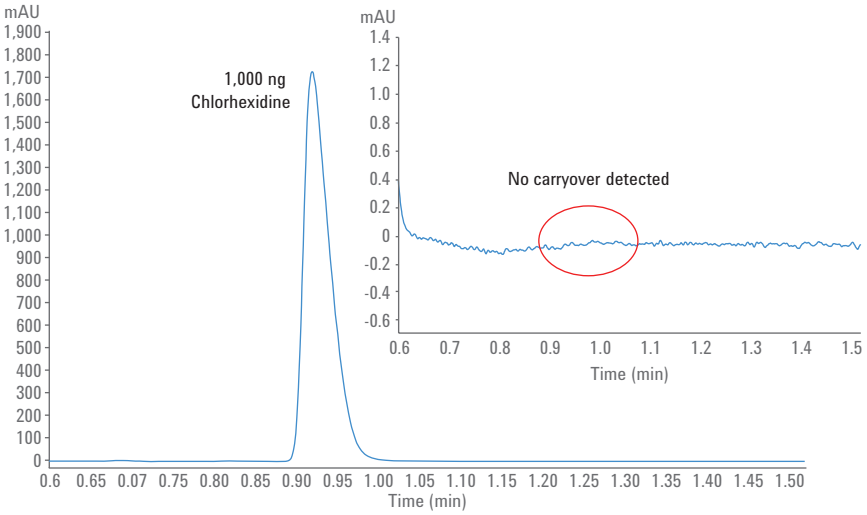

| Peak | ID | RSD RT (%) | RSD Area (%) |
|---|---|---|---|
| 1. | Dimethylphtalate | 0.016 | 0.1321 |
| 2. | Diethylphtalate | 0.028 | 0.0864 |
| 3. | Biphenyl | 0.052 | 0.0722 |
| 4. | *o*-Terphenyl | 0.054 | 0.0746 |

Isocratic sample (p/n 01080-68707)

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent ZORBAX SB C18, 4.6 × 150 mm, 1.8 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Isocratic | 70 %B |
| Flow rate | 1.2 mL/min |
| Stop time | 9 minutes |
| Injection volume | 5 µL, draw speed 200 µL/min |
| Column temperature | 40 °C |
| DAD | 254/40 nm; Ref 380/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.025 minutes, (10 Hz) |

Figure 8. Area precision for isocratic runs with 5 µL injection volume.




Sample: Chlorhexidine
1 mg/mL dissolved in 0.1 % TFA in $H_2O_{dd}$

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent Poroshell 120 EC C18, 4.6 mm × 50 mm |
| Mobile phase | A) 0.1 % TFA in in $H_2O_{dd}$<br>B) 0.1 % TFA in Acetonitrile |
| Isocratic | 40 %B |
| Flow rate | 0.6 mL/min |
| Stop time | 2.5 minutes |
| Injection volume | 1 µL, draw speed 20 µL/min 10-second needle wash (exterior) |
| Column temperature | 50 °C |
| DAD | 254/4 nm; Ref 360/100 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.0125 minutes, (20 Hz) |

Figure 9. No carryover was detected after injection of 1,000 ng chlorhexidine.

## Injector performance – injection volume linearity

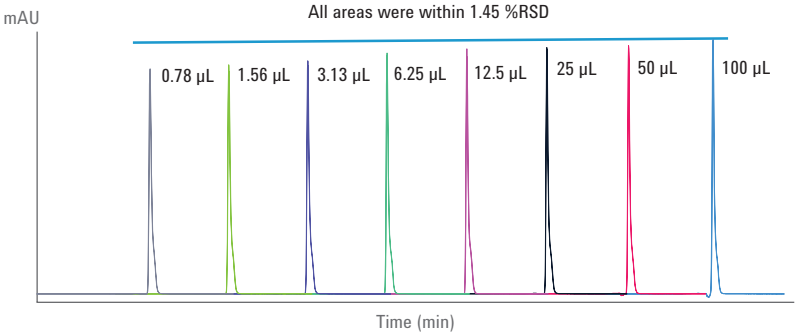
Injection volume linearity was tested using caffeine standards. All injection volumes contained 781.26 ng of caffeine. As a result, the injection volume was varied, but the injected amount always remained the same (Figure 10). The peak heights and areas should be the same for all injection volumes. The experiments showed that all areas were within 1.45 % RSD over the complete injection volume range of 0.78 to 100 µL for n = 5.

## Performance of the Agilent 1260 Infinity II DAD WR

The 1260 Infinity II DAD Wide Wavelength Range (WR) with the bio-inert standard flow cell is recommended for all common applications.

## Detector performance – linearity

Linearity was tested using caffeine standards from 1.5 to 1,600 ng injected with n = 5. Good linearity was obtained at this concentration range. The coefficient of correlation was 0.99995. The response factors were within the 5 % error range over an absorbance range of 1.7 to 2,536 mAU (Figure 11).

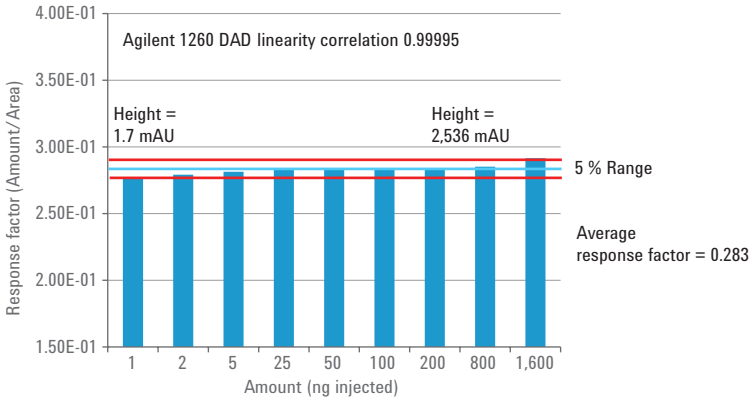



Sample: Caffeine 1 mg/mL,
7 times 1:2 diluted

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 1.8 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Isocratic | 30 %B |
| Flow rate | 0.8 mL/min |
| Stop time | 2.5 minutes |
| Injection volume | 0.78 to 100 µL, draw speed 50 µL/min |
| Column temperature | 50 °C |
| DAD | 254/4 nm; Ref 380/80 nm |
| Flow cell | Bio-inert standard flow cell, 10 mm |
| Peak width | <0.0125 minutes, (20 Hz) |

Figure 10. Injection volume linearity from 0.78 up to 100 µL; injected amount was always the same.




Sample: Enterprise Edition Caffeine
Extended Standards Kit

**Chromatographic conditions**

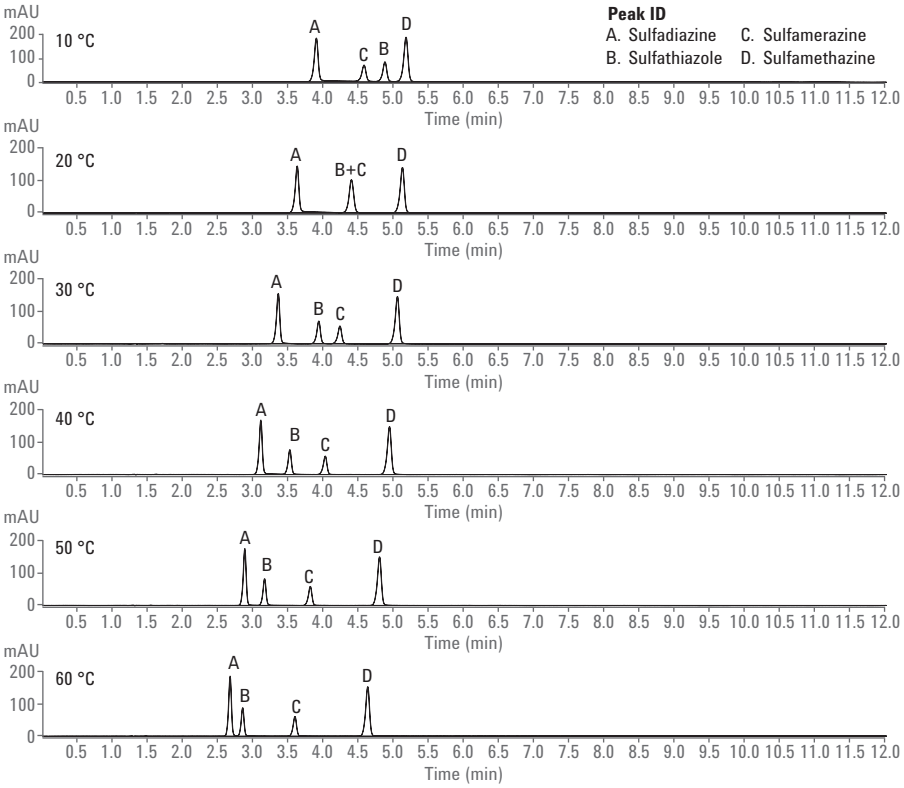
| | |
|---|---|
| Column | Agilent Poroshell 120 EC C18, 3.0 × 50 mm, 1.9 µm |
| Mobile phase | A) Water, B) Acetonitrile |
| Isocratic | 10 %B |
| Flow rate | 0.8 mL/min |
| Stop time | 2.5 minutes |
| Injection volume | 1 µL, 2 µL (1,600 ng) |
| Column temperature | 30 °C |
| DAD | 273/10 nm; Ref 380/100 nm |
| Flow cell | Universal bio-inert standard flow cell, 10 mm |
| Peak width | <0.0125 minutes, (20 Hz) |

Figure 11. Linearity of the Agilent 1260 Infinity II DAD WR with bio-inert flow cell.

## Multicolumn thermostat – comparison of standard and bio-inert heat exchangers

The column temperature regulation using the bio-inert heat exchanger was compared to the standard (stainless steel) heat exchanger in the MCT. Samples were sensitive to temperature changes regarding RT (sulfa drugs: sulfadiazine,sulfathiazole,sulfamerazine, and sulfamethazine).

With the change of solvent temperature, component B (sulfathiazole) changed position within the order of eluted components during the isocratic run. At 10 °C, component B eluted at the third position, whereas at 30 °C and above it eluted at the second position using the standard heat exchanger (Figure 12).



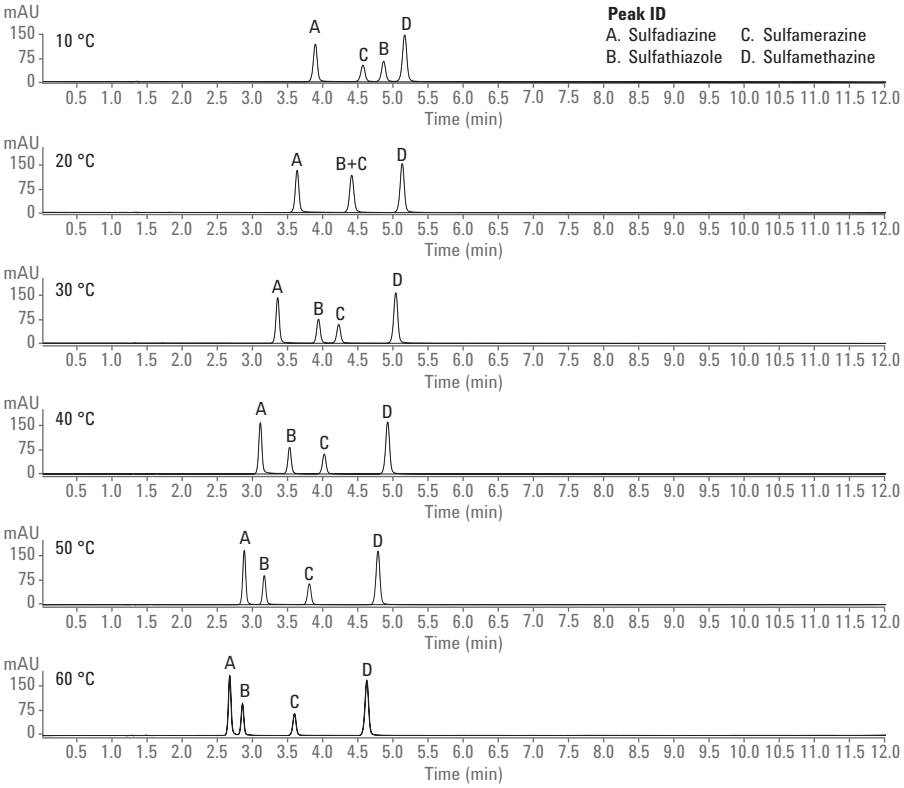

**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent InfinityLab, EC 120, 4.6 × 100 mm, 2.7 µm |
| Mobile phase | A) Water + 0.1 % TFA<br>B) Acetonitrile + 0.1 % TFA |
| Flow rate | 1 mL/min |
| Gradient | 0 minutes, 10 %B<br>10 minutes, 25 %B<br>12 minutes, 10 %B |
| Stop time | 12.01 minutes |
| Post time | 5 minutes |
| Column temperature | 60 °C, 50 °C, 40 °C, 30 °C, 20 °C, 10 °C |
| DAD | 254/4 nm; Ref 380/100 nm |
| Flow cell | bio-inert standard flow cell, 10 mm |
| Peak width | <0.0125 minutes, (20 Hz) |

Figure 12. Thermosensitive sulfa drugs, with column temperature regulated through the standard heat exchanger.

Using the bio-inert heat exchanger, no major differences have been seen regarding RT changes (Figure 13).

Table 1 shows the relative RT of sulfathiazole (component B) referred to sulfamerazine (component C) with n = 3. Both heat exchangers are highly comparable.




**Chromatographic conditions**

| | |
|---|---|
| Column | Agilent InfinityLab, EC 120, 4.6 × 100 mm, 2.7 µm |
| Mobile phase | A) Water + 0.1 % TFA<br>B) Acetonitrile + 0.1 % TFA |
| Flow rate | 1 mL/min |
| Gradient | 0 minutes, 10 %B<br>10 minutes, 25 %B<br>12 minutes, 10 %B |
| Stop time | 12.01 minutes |
| Post time | 5 minutes |
| Column temperature | 60 °C, 50 °C, 40 °C, 30 °C, 20 °C, 10 °C |
| DAD | 254/4 nm; Ref 380/100 nm |
| Flow cell | bio-inert standard flow cell, 10 mm |
| Peak width | <0.0125 minutes, (20 Hz) |

Figure 13. Thermosensitive sulfa drugs, with column temperature regulated through the bio-inert heat exchanger.

Table 1. Comparison of standard and bio-inert heat exchanger.

| Relative RT of sulfathiazole (component B) referred to sulfamerazine (component C) | | | | | | |
|---|---|---|---|---|---|---|
| Standard heat exchanger | –18 | 0 | +18 | +30 | +39 | +45 |
| Bio-inert heat exchanger | –18 | 0 | +17 | +30 | +39 | +44 |

## Conclusions

The performance of the Agilent 1260 Infinity II Bio-inert LC meets the requirements of modern analytical liquid chromatography. The performance is highly comparable with the Agilent 1260 Infinity Bio-inert Quaternary LC. The 1260 Infinity II Bio-inert LC is well suited for 4.6, 3.0, and 2.1 mm id columns, and can be used for conventional HPLC and UHPLC on columns packed with 1.8 µm particles. Precision of RTs for conventional LC is typically <0.04 % RSD. The precision for peak areas is typically <0.14 % for injection volumes >5 µL. No carryover was detected after 1,000 ng chlorhexidine injection.

The Agilent 1260 Infinity II DAD WR with a Bio-inert standard flow cell is recommended for all common applications. It is especially well suited for bio-analytical applications, for example, protein analysis using ion exchange chromatography (IEX) or size exclusion/gel filtration (SEC). Low-pressure applications around 21 bar, commonly used in protein analysis and analytical scale preparative LC, showed high RT precision.

## References


1. Schneider, S. Performance characteristics of the 1260 Infinity Bio-inert Quaternary LC System, Agilent Technologies Technical Overview, publication number 5990-9194EN, 2011.

2. Agilent 1260 Infinity Bio-inert Quaternary LC, Features, Technical Details, Applications, and Specifications, Agilent Technologies Data Sheet, publication number 5990-6129EN, 2012.



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