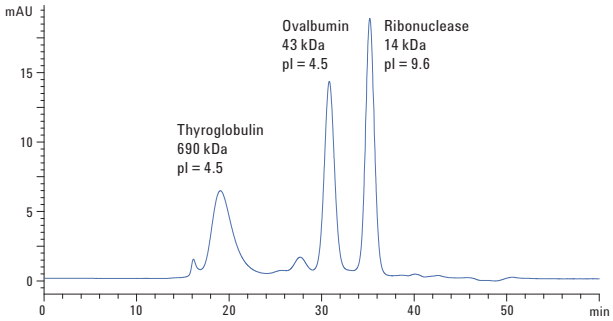
Proof of Performance

Performance characteristics of the 1260 Infinity Bio-inert Quaternary LC System

Technical Overview



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Abstract

The Agilent 1260 Infinity Bio-inert Quaternary LC system has the same performance specifications as the standard Agilent 1260 Infinity Quaternary LC system based on the proven technology of the Agilent 1200 Infinity Series. This Technical Overview proves that method transfer from an Agilent 1260 Infinity Series Quaternary LC system to an Agilent 1260 Infinity Bio-inert Quaternary LC system will typically result in chromatograms with no significant differences including compatibility with standard methods with a maximum pressure of 600 bar. In addition, it is especially well suited for bio-analytical applications, such as, protein analysis using ion exchange chromatography (IEX) or size exclusion/gel filtration (SEC).

Agilent Technologies

Introduction

The Agilent 1260 Infinity Bio-inert Quaternary LC system was especially designed for conditions generally used in bio-chromatography – for example, high salt concentrations (2 M NaCl, up to 8 M urea), high and low pH solvents (0.5 M NaOH, 0.5 M HCl) – by deploying with a completely inert sample flow path. All capillaries and fittings throughout the autosampler, column compartment, and detectors are completely metal-free so that biomolecules come in contact only with ceramics or PEEK. Based on the proven technology of the Agilent Infinity Series liquid chromatography platform, the 1260 Infinity Bio-inert Quaternary LC, has the same performance specifications as the standard 1260 Infinity Quaternary LC system[1] – and is compatible with standard methods with a maximum pressure of 600 bar.

The Agilent 1260 Infinity Bio-inert Quaternary LC system offers:

- Flow rate range up to 5 mL/min (required for UHPLC on standard bore columns with 3–4.6 mm id)
- Space for long HPLC columns (250–300 mm) in the thermostatted column compartment
- Maximum pressure of 600 bar for UHPLC on columns up to 150 mm length. Higher pressures allow support of either higher flow rates (more speed) or longer columns (more resolution).
- Maximum temperature of 80 °C (100 °C with an Agilent 1290 Infinity Thermostatted Column Compartment). Higher temperatures reduce pressures significantly, thereby allowing higher flow rates for more speed on longer columns.
- Agilent 1260 Infinity LC Diode Array Detector VL (G1315D) with standard flow cell (10 mm)
- Agilent 1260 Infinity LC Diode Array Detector (G4212B) with a new optical design, which uses a cartridge cell with optofluidic waveguide technology offering better sensitivity with low dispersion
- Standard bore UHPLC and conventional applications run on the same system configuration

Experimental

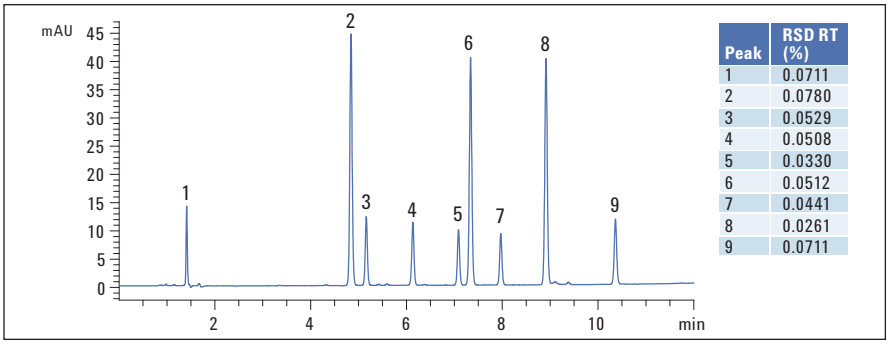
The Agilent 1260 Infinity Bio-inert Quaternary LC system, that was tested, consisted of:

- Agilent 1260 Infinity Bio-inert Quaternary Pump (G5611A)
- Agilent 1260 Infinity High Performance Bio-inert Autosampler (G5667A)
- Agilent 1260 Infinity DAD (G4212B) with bio-inert Max-Light cartridge cell, 10 mm or
- Agilent 1260 Infinity DAD VL (G1315D) with bio-inert standard flow cell, 10 mm
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Software: OpenLAB CDS, ChemStation Edition for LC & LC MS Systems, Rev. C.01.02 [14]

1. Pump performance

1A. Retention time precision

The most important parameter influencing retention time precision is pump performance. Retention time precision was tested with different gradient and isocratic conditions using 4.6 and 3 mm id columns. The relative standard deviation of retention times for conventional gradient runs was < 0.08 % RSD (Figure 1) for n = 7.



Peak	RSD RT (%)
1	0.0711
2	0.0780
3	0.0529
4	0.0508
5	0.0330
6	0.0512
7	0.0441
8	0.0261
9	0.0711

Chromatographic conditions

Sample from Sigma Aldrich:

Reversed phase test mix, (Order no.: 47641-U)
1 × 1 mL (1 uracil, 2 phenol, 6 n,n-diethyl-m-toluamide, 8 toluene)

HPLC Gradient system diagnostic mix, (Order no.: 48271)

6 × 1 mL (2 phenol, 3 methyl parabens, 4 ethyl parabens, 5 propyl parabens, 7 butyl parabens, 9 heptyl parabens, 1 uracil)

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

Sample preparation:

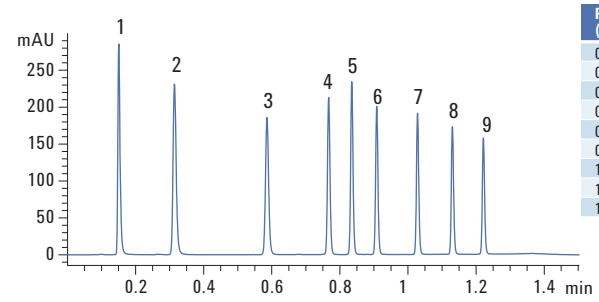
Dilute each sample to 5 mL with water/acetonitrile 1:1

Mix the two diluted samples 1:1

Figure 1
Precision of retention times for conventional gradient runs.

For fast gradients with a run time of about 1 minute, the relative standard deviation for retention times was < 0.11% RSD (Figure 2) for n = 7.

Figure 3 shows conventional isocratic conditions with a retention time precision of < 0.1% RSD for n = 7.

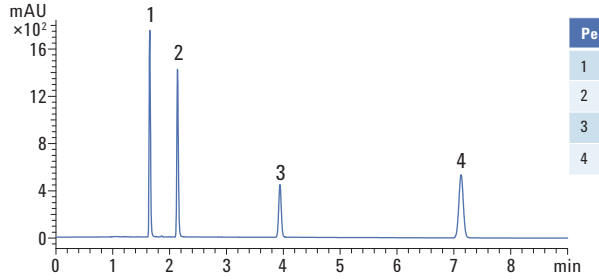


Peak RT (min)	SD (min)	RSD RT (%)
0.151	0.00016	0.108
0.314	0.00016	0.050
0.585	0.00026	0.044
0.767	0.00062	0.081
0.835	0.00066	0.079
0.909	0.0008	0.088
1.029	0.00054	0.052
1.131	0.00072	0.064
1.220	0.00126	0.103

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
	1 acetanilide 2 acetophenone 3 propiophenone 4 butyrophenone 5 benzophenone 6 valerophenone 7 hexanophenone 8 heptanophenone 9 octanophenone
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water B = Acetonitrile
Gradient:	0 min 30% B, 1 min 95% B
Flow rate:	3.5 mL/min
Stop time:	1.5 min
Post time:	1 min
Injection volume:	1 µL
Column temperature:	50 °C
DAD:	245/10 nm Ref 400/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.00625 min (40 Hz)

Figure 2
Precision of retention times for fast gradient runs.



Peak	RSD RT (%)	RSD Area (%)
1	0.07387	0.14263
2	0.08153	0.05952
3	0.07279	0.0511
4	0.08895	0.05467

Chromatographic conditions

Sample:	Isocratic sample (p/n 01080-68707)
	1 dimethylphtalate 2 diethylphtalate 3 biphenyl 4 o-terphenyl
Column:	Agilent ZORBAX SB C18, 4.6 mm × 150 mm, 1.8 µm
Mobile phase:	A = Water B = Acetonitrile
Isocratic:	70% B
Flow rate:	1.2 mL/min
Stop time:	9 min
Injection volume:	5 µL, draw speed 200 µL/min
Column temperature:	40 °C
DAD:	254/40 nm, Ref 380/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.025 min, (10 Hz)

Figure 3
RT precision for conventional isocratic runs.

1B. Performance of step gradient

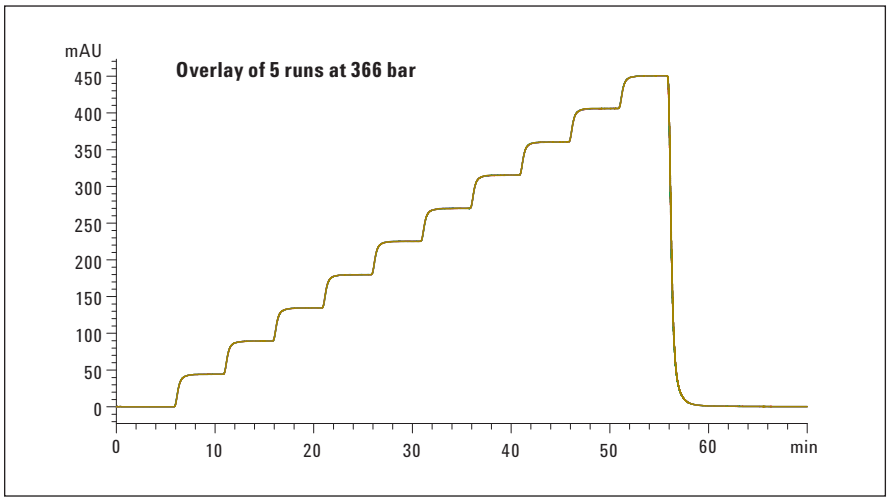
Tracer experiments are frequently used to verify the solvent mixing ripple at different gradient mixtures to evaluate pump performance. The delay volume, accuracy, and precision of gradients are also evaluated using step gradients. Figure 4 shows a step gradient from 0% to 100% in 10% steps. Caffeine was selected as the tracer compound.

The performance results are:

- Ripple on 10% step = 0.07%
- Ripple on 50% step = 0.06%
- Ripple on 90% step = 0.07%
- Precision of step height for 50% step = 0.03% RSD for 5 runs
- System delay volume: 1,000 µL at 366 bar

1C. Step gradient - Comparing 1260 Infinity LC and 1260 Infinity Bio-inert LC systems

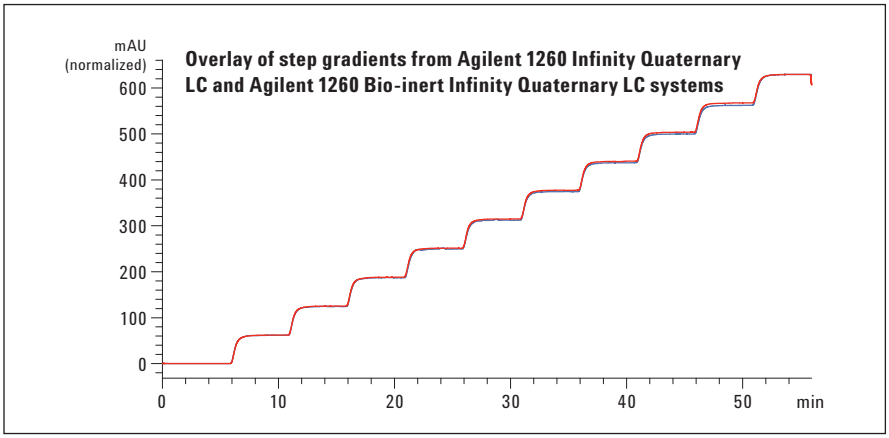
Figure 5 shows an overlay of step gradients generated with the Agilent 1260 Infinity Quaternary LC and the Agilent 1260 Infinity Bio-inert Quaternary LC systems. The performance of both systems is highly comparable regarding mixing properties of the pump.



Chromatographic conditions

Column:	Restriction capillary	Post time:	5 min
Mobile phase:	A = Water + 20% Isopropanol B = Water + 20% Isopropanol +10 mg/L caffeine	Column temperature:	36 °C
		DAD:	273/4 nm, Ref 380/100 nm
Step gradient:	From 0 to 100% B in 10% steps	Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Flow rate:	1 mL/min		
Stop time:	70 min	Peak width:	<0.0125 min, (20 Hz)

Figure 4
Different zones in the thermostatted column compartment to pre- and post-condition mobile phase temperatures.



Chromatographic conditions

Column:	Restriction capillary	Post time:	5 min
Mobile phase:	A = Water + 20% Isopropanol B = Water + 20% Isopropanol + 10 mg/L caffeine	Column temperature:	36 °C
		DAD:	273/4 nm, Ref 380/100 nm
Step gradient:	From 0 to 100% B in 10% steps	Flow cells:	Agilent standard bio-inert flow cell, 10 mm and Agilent bio-inert Max-Light cartridge cell,10 mm
Flow rate:	1 mL/min		
Stop time:	70 min	Peak width:	<0.0125 min, (20 Hz)

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

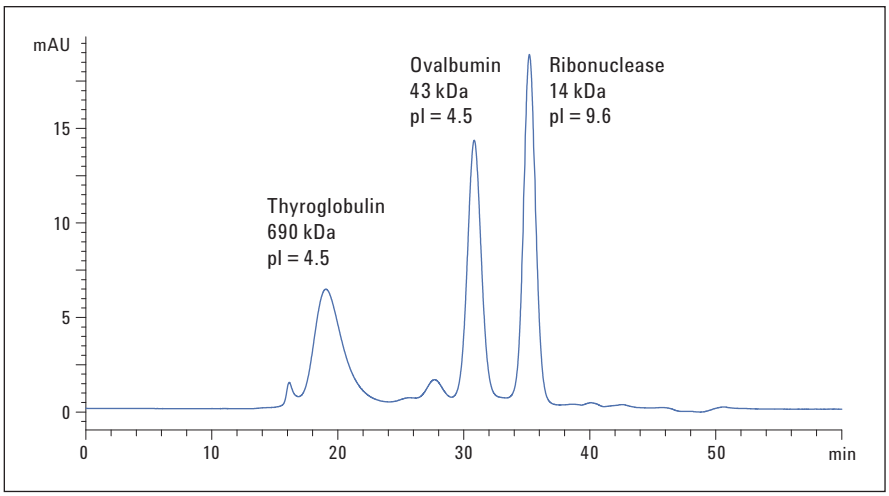
1D. Low back pressure application - Protein separation via size exclusion chromatography at 15 bar

Low back pressure applications, as commonly used in protein analysis, were tested with the Agilent 1260 Infinity Bio-inert LC system to prove pressure stability and precision of RT with the 600 bar system. High precision of retention time was obtained (RSD of <0.025%, n = 5) together with a very small pressure ripple (<0.01%). Figure 6 shows a separation of three standard proteins using a SEC column with a very low backpressure, resulting in a total system pressure of 15 bar.

2. Injector performance

2A. Area precision

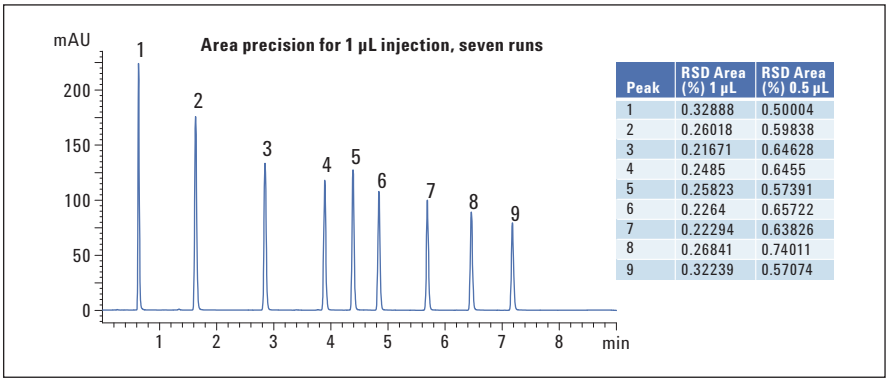
The Agilent 1260 Infinity High Performance Autosampler can inject precisely over an injection range of 0.5 to 100 µL. Figure 7 shows an example chromatogram for an injection volume of 1 µL. The relative standard deviation is <0.33 %. The relative standard deviation for an injection volume of 0.5 µL is <0.75% for n = 7.



Chromatographic conditions

Column:	SEC column
	10 × 300 mm, 13 µm
Mobile phase:	A = 150 mM Sodiumphosphate, 150 mM NaCl, pH = 7.0
Isocratic:	100% A
Flow rate:	0.5 mL/min
Column temperature:	RT
DAD:	280/4 nm, Ref 360/100 nm
Flow cell:	Agilent bio-inert standard flow cell, 10 mm
Peak width:	<0.10 min, (2.5 Hz)

Figure 6
Low back pressure application - separation of a protein mix using SEC at 15 bar.



Peak	RSD Area (%) 1 µL	RSD Area (%) 0.5 µL
1	0.32888	0.50004
2	0.26018	0.59838
3	0.21671	0.64628
4	0.2485	0.6455
5	0.25823	0.57391
6	0.2264	0.65722
7	0.22294	0.63826
8	0.26841	0.74011
9	0.32239	0.57074

Chromatographic conditions

Sample:	RRLC Checkout sample (p/n 5188-6529)
1 acetanilide	6 valerophenone
2 acetophenone	7 hexanophenone
3 propiophenone	8 heptanophenone
4 butyrophenone	9 octanophenone
5 benzophenone	
Column:	Agilent Poroshell 120 EC C18, 3 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water, B = Acetonitrile
Gradient:	0 min 20% B, 8 min 80% B
Flow rate:	1.2 mL/min
Stop time:	8 min
Post time:	4 min
Injection volume:	1 µL
Column temperature:	30 °C
DAD:	245/10 nm, Ref 400/100 nm
Flow cell:	Agilent Bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.025 min (10 Hz)

Figure 7
Area precision for conventional runs for 1 µL.

Figure 8 shows an example chromatogram for an injection volume of 5 µL. The relative standard deviation is <0.1% for n = 7.

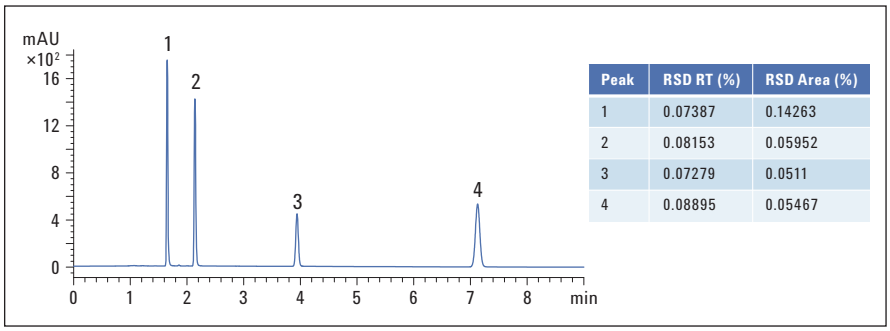
The injector settings are very important for optimum precision of areas. For example, if the highest precision is needed, the draw speed of the injector should be set to lower values, especially if large volumes or viscous samples are injected. It is also important to avoid solvent evaporation from the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

2B. Carryover

For the injection, the draw speed was set to 20 µL/min and an exterior needle wash for 10 sec was used. The carryover (Figure 9) was found to be <0.0011% (11 ppm) for the conditions used, which is in the specification range[2]. After a 1,000-ng sample injection, unadulterated solvent was injected.

2C. 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 also highly recommended to reconnect the capillary connections from time to time to prevent cavities, which can lead to enhanced carryover.

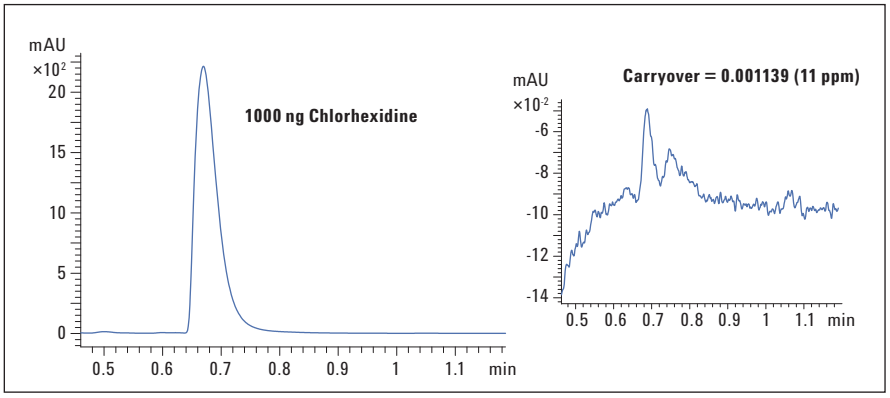


Peak	RSD RT (%)	RSD Area (%)
1	0.07387	0.14263
2	0.08153	0.05952
3	0.07279	0.0511
4	0.08895	0.05467

Chromatographic conditions

Sample:	Isocratic sample (p/n 01080-68707) 1 dimethylphtalate 2 diethylphtalate 3 biphenyl 4 o-terphenyl
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 min
Injection volume:	5 µL, draw speed 200 µL/min
Column temperature:	40 °C
DAD:	254/40 nm, Ref 380/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.025 min, (10 Hz)

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



Chromatographic conditions

Sample:	Chlorhexidine 1,000 ng/µL dissolved in 0.1% TFA in H_20_{dd}
Column:	Agilent Poroshell 120 EC C18, 3 mm x 50 mm
Mobile phase:	A = 0.1% TFA in in H_20_{dd} B = 0.1% TFA in Acetonitrile
Isocratic:	40% B
Flow rate:	0.6 mL/min
Stop time:	2.5 min
Injection volume:	1 µL, draw speed 20 µL/min 10 s needle wash (exterior)
Column temperature:	50 °C
DAD:	254/4 nm, Ref 360/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 9
Carryover = 0.001139% after injection of 1,000 ng Chlorhexidine.

2D. Injection volume linearity

Injection volume linearity was tested using Primidone standards. All injection volumes contained 781.26 ng of Primidone. This means the injection volume was varied but the injected amount remained always 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 2.26% RSD over the complete injection volume range of 0.8 to 100 µL for n = 5.

3. Detector performance

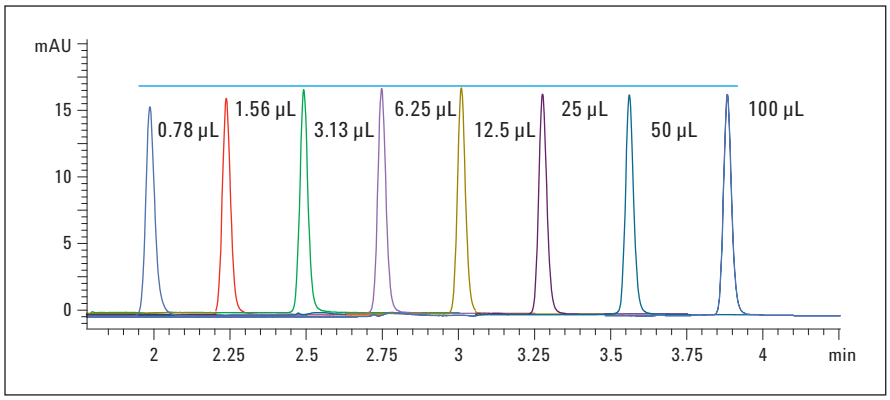
The performance was tested with two different DADs:

- Agilent 1260 Infinity DAD (G4212B) with the bio-inert Agilent Max Light cartridge flow cell, 10 mm
- Agilent 1260 Infinity DAD VL (G1315D) with the bio-inert standard flow cell, 10 mm

3A. Baseline ASTM noise and drift

The ASTM noise and drift were evaluated using a restriction capillary and water as mobile phase. The detector was set to a 4-second response time. The resulting ASTM noise for the 1260 Infinity DAD was found to be at ± 3.28 µAU and the drift was –45 µAU. The 1260 Infinity DAD VL showed an ASTM noise ± 9.38 µAU and a drift of –6.75 µAU.

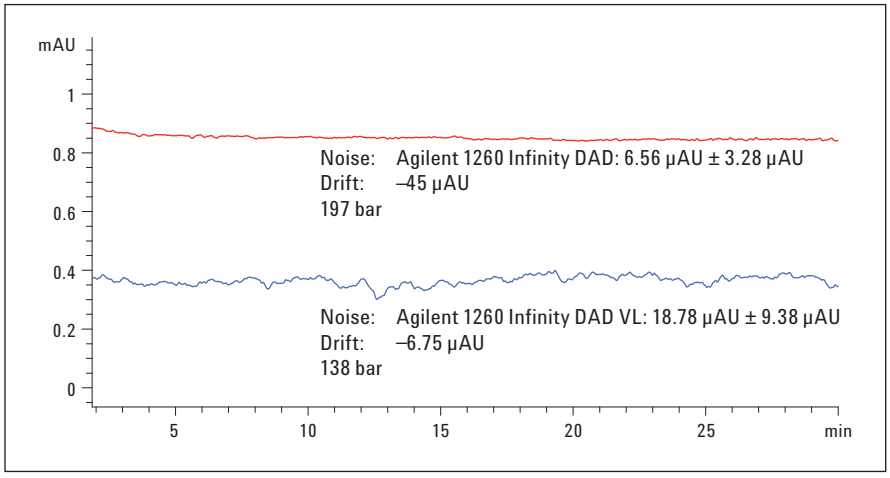
Figure 11 shows an example chromatogram for the noise and drift for both detectors. The Agilent 1260 Infinity DAD VL with the bio-inert standard flow cell is recommended for all common applications whereas the Agilent 1260 Infinity DAD with the bio-inert Max-Light cartridge flow cell can be particularly used for high sensitivity applications due to the lower noise of the cell. For cation exchange chromatography and high salt size



Chromatographic conditions

Sample:	Primidone 1 mg/mL 7 times 1:2 diluted
Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100, 1.8 µm
Mobile phase:	A = Water, B = Acetonitrile
Isocratic:	30% B
Flow rate:	0.8 mL/min
Stop time:	2.5 min
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:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 10
Injection volume linearity from 0.8 up to 100 µL; with consistent injected amount.



Chromatographic conditions

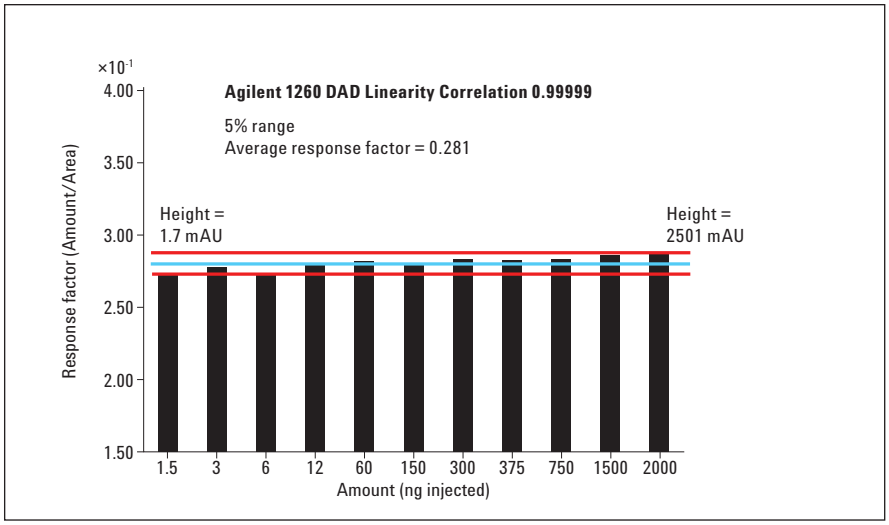
Column:	Restriction capillary with 197 or 138 bar back pressure
Mobile phase:	A = Water
Flow rate:	1 mL/min
Stop time:	30 min
Column temperature:	36 °C
DAD:	254/4 nm, Ref 380/80 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm and Agilent bio-inert standard flow cell, 10 mm
Peak width:	<0.2 min, (1.25 Hz)

Figure 11
Noise and drift for the Agilent 1260 Infinity DAD with bio-inert Max-Light cartridge flow cell and the Agilent 1260 Infinity DAD VL with bio-inert standard flow cell.

exclusion chromatography (SEC) applications the 1260 Infinity DAD VL with the bio-inert standard flow cell is highly recommended.

3B. Linearity

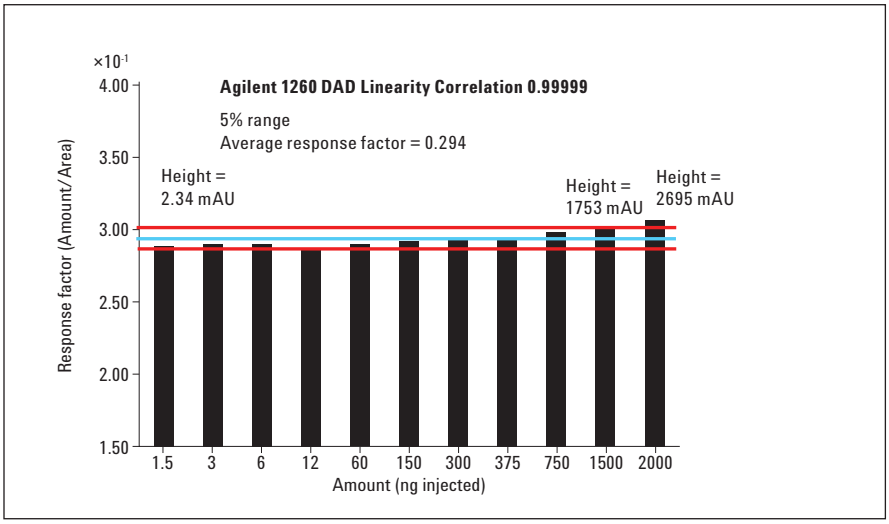
The linearity was tested with both DADs with bio-inert flow cells using caffeine standards from 1.5 ng to 2,000 ng injected amount with an n = 5. For this concentration range, very good linearity was obtained. The coefficient of correlation was 0.99999. The response factors were within the 5% error range over an absorbance range of 1.7 to 2501 mAU for the 1260 Infinity DAD VL with bio-inert standard flow cell (Figure 12), and of 2 up to 1,753 mAU for the 1260 Infinity DAD with bio-inert 10 mm Agilent Max-Light cartridge flow cell (Figure 13).



Chromatographic conditions

Sample:	Caffeine
Column:	Agilent Poroshell 120 EC C18, 3.0 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water, B = Acetonitrile
Isocratic:	10% B
Flow rate:	0.8 mL/min
Stop time:	2.5 min
Injection volume:	1.5 µL, 2 µL (2,000 ng)
Column temperature:	30 °C
DAD:	273/10 nm, Ref 380/800 nm
Flow cell:	Agilent bio-inert standard flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 12
Linearity of the Agilent 1260 Infinity DAD VL with Agilent bio-inert standard flow cell.



Chromatographic conditions

Sample:	Caffeine
Column:	Agilent Poroshell 120 EC C18, 3.0 mm × 50 mm, 2.7 µm
Mobile phase:	A = Water, B = Acetonitrile
Isocratic:	10% B
Flow rate:	0.8 mL/min
Stop time:	2.5 min
Injection volume:	1.5 µL, 2 µL (2,000 ng)
Column temperature:	30 °C
DAD:	273/10 nm, Ref 380/800 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 13
Linearity of the Agilent 1260 Infinity DAD with Agilent bio-inert Max-Light cartridge flow cell, 10 mm.

3C. Sensitivity for anthracene

The sensitivity of the Agilent 1260 Infinity DAD and the Agilent 1260 Infinity DAD VL was compared by determining LOD and LOQ using anthracene (2.5 ng/µL in acetonitrile) as sample with n = 7. The signal-to-noise ratios as well as LOD and LOQ were in a similar range using the Agilent 1260 Infinity DAD with standard and bio-inert Max-Light cartridge flow cells, with 10 mm path length (Figure 14). Due to a higher noise range of the Agilent 1260 Infinity DAD VL with the bio-inert standard flow cell (10 mm) LOD and LOQ are increased by a factor of four.

Bio-inert standard flow cell, 10 mm		
S/N	LOD (pg/µL)	LOQ (pg/µL)
341	21.99	65.98
Bio-inert Max-Light cartridge flow cell, 10 mm		
S/N	LOD (pg/µL)	LOQ (pg/µL)
1225	6.12	18.37
Max-Light cartridge flow cell, 10 mm		
S/N	LOD (pg/µL)	LOQ (pg/µL)
1543	4.86	14.58

Chromatographic conditions

Sample:	Anthracene 2.5 ng/µL	Stop time:	4.5 min
Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 × 1.8 µm	Post time:	1.5 min
		Injection volume:	1 µL
Mobile phase:	A = Water, B = Acetonitrile	Column temperature:	25 °C
Gradient:	0 min 40% B, 3 min 95% B	DAD:	254/10 nm, Ref 400/80 nm
Flow rate:	1.5 mL/min	Peak width:	<0.025 min, (10 Hz)

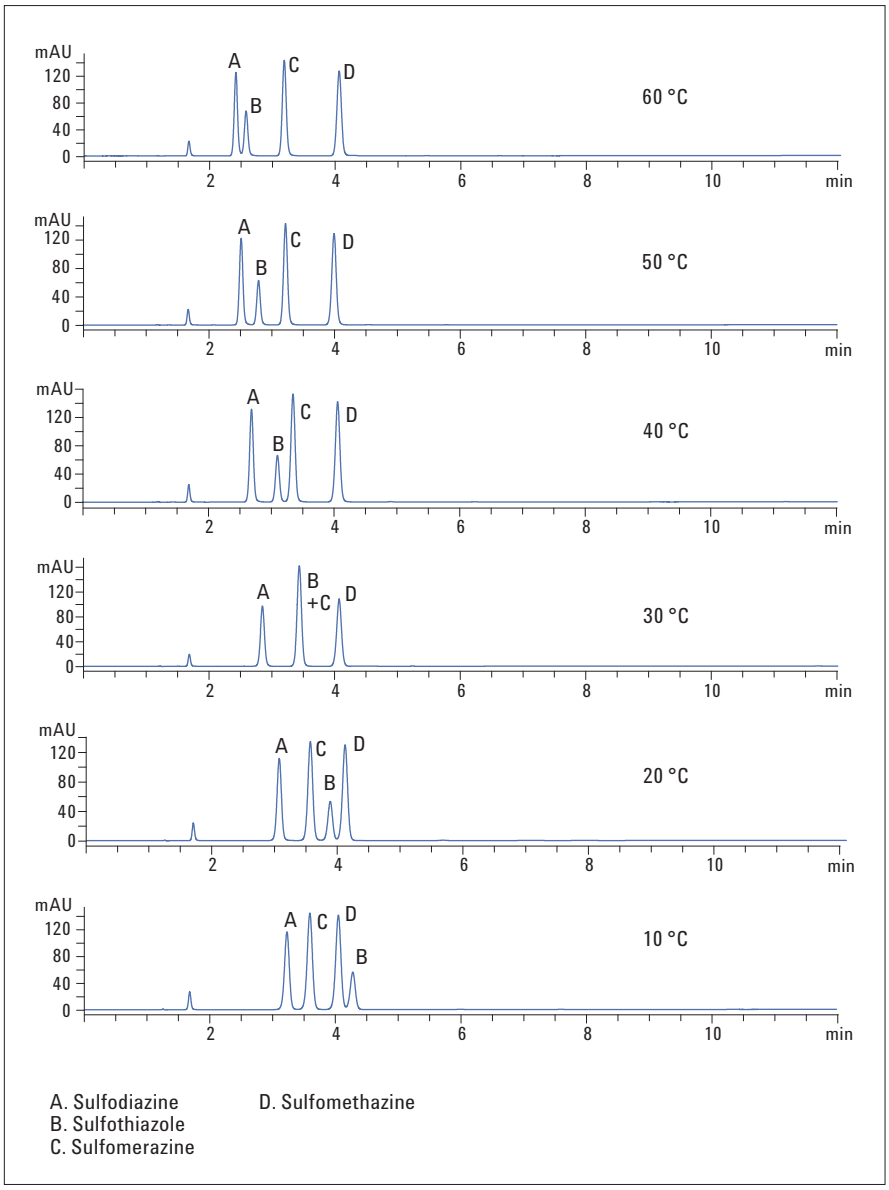
Figure 14
Sensitivity of Agilent 1260 Infinity DAD and Agilent 1260 Infinity DAD VL with bio-inert and standard flow cells, 10 mm.

4. Thermostatted column compartment performance

Comparing standard and bio-inert heat exchanger

The column temperature regulation using the bio-inert heat exchanger was compared to the standard thermostatted column compartment (TCC) using samples that are sensitive to temperature changes regarding retention time (sulfadrugs: sulfadiazine, sulfathiazole, sulfamerazine, and sulfamethazine).

With the change of solvent temperature component B (sulfothiazole) changed position within the order of eluted samples during the isocratic run. At 60 °C, component B eluted on second position, whereas at 10 °C it eluted on fourth position using the standard TCC (Figure 15).

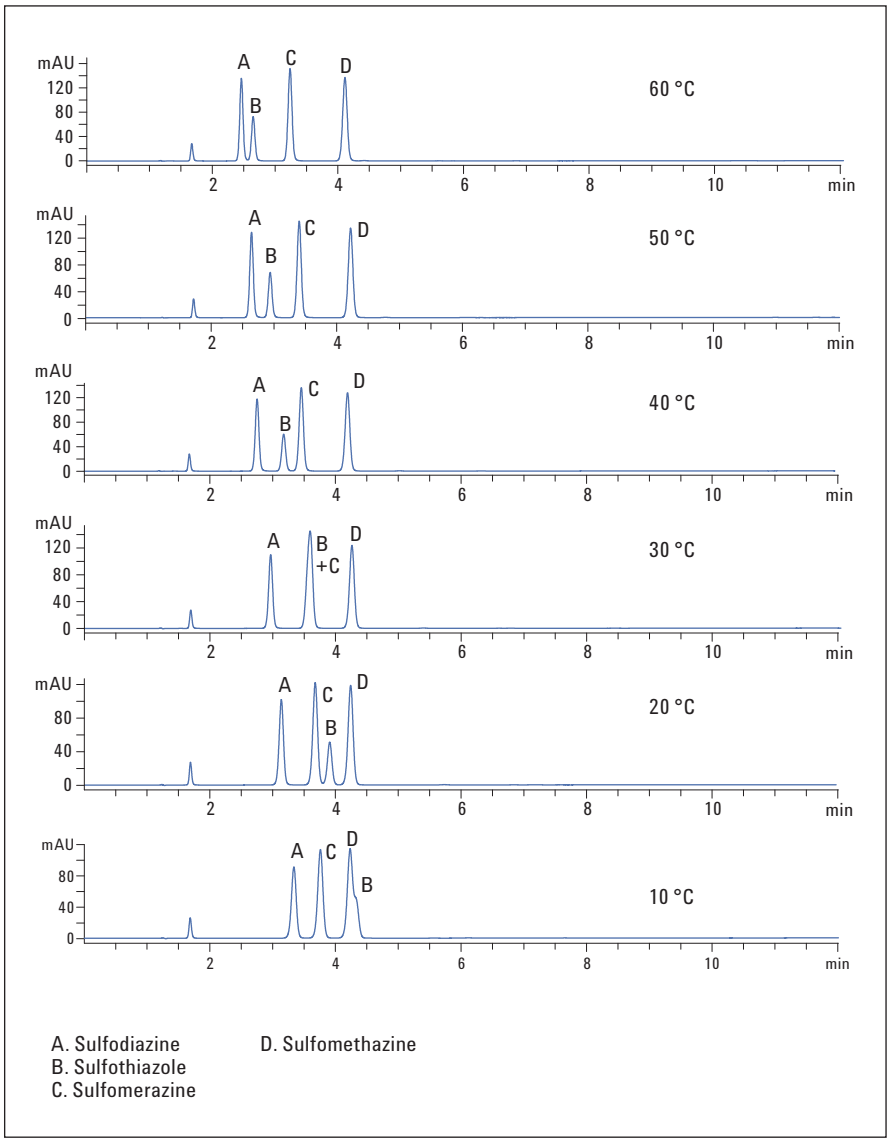


Chromatographic conditions

Column:	Agilent Eclipse Plus, 4.6 × 100 × 5 µm
Mobile phase:	A = Water + 0.1% TFA B = Acetonitrile + 0.1% TFA
Flow rate:	1 mL/min
Gradient:	0 min 10% B 10 min 25% 12 min 10% B
Stop time:	12.01 min
Post time:	5 min
Column temperature:	60 °C, 50 °C, 40 °C, 30 °C, 20 °C, 10 °C
DAD:	254/4 nm, Ref 380/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 15
Thermosensitive sulfadrugs, column temperature regulated via standard TCC.

Using the bio-inert heat exchanger component B is only identifiable as a shoulder on the third peak (component D) at 10 °C (Figure 16).



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DAD:	254/4 nm, Ref 380/100 nm
Flow cell:	Agilent bio-inert Max-Light cartridge flow cell, 10 mm
Peak width:	<0.0125 min, (20 Hz)

Figure 16
Thermosensitive sulfadrugs, column temperature regulated via bio-inert heat exchanger.

Table 1 shows the relative retention time of sulfathiazole (component B) referred to sulfamerazine (component C) with n = 3. With the standard TCC the movement of component B through the chromatogram was expanded compared to the movement using the bio-inert heat exchanger. The different composition of the bio-inert heat exchanger, containing PEEK on the inside, results in different heat transfer/conductivity. This fact is to be considered when transferring standard Agilent 1260 Infinity Quaternary LC methods to the bio-inert system.

	Relative RT of sulfathiazole (component B) referred to sulfamerazine (component C)					
Standard TCC	–19	–13	–7	0	9	19
Bio-inert TCC	–18	–13	–8	0	6	15

Table 1
Comparison between standard TCC and bio-inert heat exchanger.

Conclusion

The performance of the Agilent 1260 Infinity Bio-inert Quaternary LC system meets the requirements of modern analytical liquid chromatography.

It is well suited for 3 and 4.6 mm id columns, and can be used for conventional HPLC and UHPLC on columns packed with 1.8 µm particles. Precision of retention times for conventional LC is typically < 0.08% RSD. The precision for areas is typically < 0.15% for injection volumes > 5 µL. Carryover is typically < 0.0011%. The ASTM noise for the Agilent 1260 Infinity DAD with bio-inert Agilent Max-Light cartridge flow cell was found to be at ± 3.28 µAU with a drift of –45 µAU/h. The Agilent 1260 Infinity DAD VL with bio-inert standard flow cell showed an ASTM noise of ± 9.38 µAU and a drift of –6.75 µAU/h.

The universal Agilent 1260 Infinity DAD VL with bio-inert standard flow cell (10 mm) is recommended for all common applications whereas the Agilent 1260 Infinity DAD with Agilent bio-inert Max-Light cartridge flow cell, 10 mm, can be particularly used for high sensitivity applications due to the lower noise of the cell. For all applications using proteins as sample, such as cation exchange chromatography and high salt size exclusion chromatography (SEC), the Agilent 1260 Infinity DAD VL with bio-inert standard flow cell is highly recommended.

Method transfer from an Agilent 1260 Infinity Quaternary LC system to an Agilent 1260 Infinity Bio-inert Quaternary LC system will typically result in chromatograms with no significant differences. The difference in the heat transfer of the bio-inert heat exchanger, due to the PEEK inlet, has to be taken into consideration.

In addition, 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 15 bar, commonly used in protein analysis and analytical scale preparative LC, showed high retention time precision and very low pressure ripple obtaining high sensitivity.

References

1. "Performance characteristics of the 1260 Infinity Quaternary LC system", Agilent Technologies publication number 5990-6026EN

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

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