Performance Characteristics of the Agilent 1290 Infinity II Vialsampler

Technical Overview

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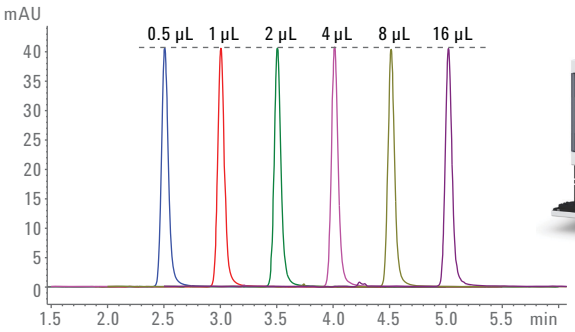
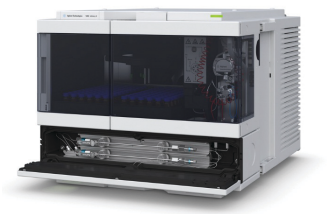
Abstract

This Technical Overview describes the features and performance of the Agilent 1290 Infinity II Vialsampler. With a power range up to 1,300 bar, and the optional integration of an integrated column compartment (ICC), the 1290 Infinity II Vialsampler provides highest flexibility, reliability, and ease-of-use. The area precision under isocratic and gradient conditions was excellent, and no carryover using caffeine and chlorhexidine was detected under the used conditions. Furthermore, the injection volume linearity showed good results for injection volumes of 0.5 to 16 µL.





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Introduction

The Agilent 1290 Infinity II Vialsampler is designed for UHPLC applications with a maximum pressure of 1,300 bar for the whole injection volume range of 0.1 to 20 µL, with a multidraw extension from 0.1 to 120 µL using the standard 40-µL analytical head. The 1290 Infinity II Vialsampler uses vial gripper robotics for fast movement of vials from the trays to the point of injection. This enables fast injection cycle times of 18 seconds. The 1290 Infinity II Vialsampler provides an extended vial capacity for up to 132 2-mL vials (two 66 microtiter plates (MTPs)) or 38 6-mL vials with easy access during the sample run. In addition, an external tray extension can be used for five vials and one disposal position. For lowest carryover, the proven flow-through design is used, and a needle wash port is included. Another benefit of the 1290 Infinity II Vialsampler is the option of an integrated sample cooler (cooling down to 4 °C) and an integrated column compartment (ICC). The ICC allows column heating up to 80 °C, and has the capacity for two columns. Based on the used flow rates, two different ICCs are available. For standard flow rates, an ICC with a (nonexchangeable) 3-µL heat exchanger is recommended, and for high flow rates, greater than 2.5 mL/min, a (nonexchangeable) 6-µL heat exchanger. The 1290 Infinity II Vialsampler is a compact module with reduced system size, convenient access for columns, and excellent price-performance ratio. To demonstrate the performance characteristics, the area precision for different injection volumes under isocratic and gradient conditions, carryover, and injection volume linearity were evaluated.

Experimental

Instrumentation

The Agilent 1290 Infinity II LC system used for the experiments consisted of the following modules:

- Agilent 1290 Infinity II High-Speed Pump (G7120A)
- Agilent 1290 Infinity II Vialsampler (G7167B), equipped with an integrated column compartment (3-µL heater; G7130-64430) and an integrated sample cooler
- Agilent 1290 Infinity II Diode Array Detector (G7117B), equipped with a 10-mm Max-Light cartridge cell

Solvents and samples

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). Chlorhexidine and caffeine were purchased from Sigma-Aldrich Corp., St. Louis, USA.

The Agilent isocratic standard sample (p/n 01080-68707) consisted of four compounds (listed in elution order):

1. Dimethyl phthalate
2. Diethyl phthalate
3. Biphenyl
4. *o*-terphenyl

The Agilent RRLC checkout sample (p/n 5188-6529) consisted of nine compounds (listed in elution order):

1. Acetanilide
2. Acetophenone
3. Propiophenone
4. Butyrophenone
5. Valerophenone
6. Hexanophenone
7. Heptanophenone
8. Octanophenone
9. Benzophenone

Columns

- Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (p/n 959759-902)
- Agilent ZORBAX Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)
- Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)

Software

Agilent OpenLAB CDS ChemStation Edition for LC and LC/MS systems, Version C.01.07 [27]

Methods

Table 1. Chromatographic parameters for area precision measurements under isocratic conditions.

Parameter	Value
Sample	Isocratic standard
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase A	65 % Acetonitrile in water
Flow rate	0.500 mL/min
Stop time	3.5 minutes
Injection volume	0.5, 1, and 3 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	40 °C
Detection	254/10 nm, reference wavelength 360/100 nm, 40 Hz

Table 2. Chromatographic parameters for area precision measurements under gradient conditions.

Parameter	Value
Sample	Agilent RRLC checkout sample
Column	Agilent ZORBAX Posroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)
Mobile phase	A) Water B) Acetonitrile
Flow rate	1.2 mL/min
Gradient	20 %B to 95 %B in 5 minutes
Stop time	5.5 minutes
Post time	1 minute
Injection volume	1, 3, 5, 10, 15, and 20 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	40 °C
Detection	254/10 nm, reference wavelength 360/100 nm, 20 Hz

Table 3. Chromatographic parameters for area precision measurements under ultrafast gradient conditions.

Parameter	Value
Sample	Agilent RRLC checkout sample
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase	A) Water B) Acetonitrile
Flow rate	1.2 mL/min
Gradient	5 %B to 95% B in 0.5 minutes
Stop time	1 minute
Post time	1 minute
Injection volume	0.5 and 1 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	60 °C
Detection	254/10 nm, reference wavelength 360/100 nm, 80 Hz

Table 4. Chromatographic parameters for carryover experiments using chlorhexidine.

Parameter	Value
Sample	Chlorhexidine, 1,000 ng/µL, blanks using 0.1 % TFA in water
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase	A) 0.1 % TFA in water B) 0.1 % TFA in acetonitrile
Flow rate	1.2 mL/min
Isocratic conditions	33 % acetonitrile in water + 0.1 % TFA
Stop time	2.5 minutes
Injection volume	1 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	50 °C
Detection	257/4 nm, reference wavelength 360/100 nm, 20 Hz

Table 5. Chromatographic parameters for carryover experiments using caffeine.

Parameter	Value
Sample	Caffeine, 500 ng/µL, blanks using water
Column	Agilent ZORBAX SB-C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
Mobile phase	A) 0.1 % TFA in water B) 0.1 % TFA in acetonitrile
Flow rate	1 mL/min
Isocratic conditions	20 % acetonitrile in water + 0.1 % TFA
Stop time	5 minutes
Injection volume	5 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	40 °C
Detection	273/4 nm, reference wavelength 360/100 nm, 20 Hz

Table 6. Chromatographic parameters for volume linearity experiments.

Parameter	Value
Sample	Caffeine, 125 ng/µL diluted five times at 1:2
Column	Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
Mobile phase	A) 0.1 % TFA in water B) 0.1 % TFA in acetonitrile
Flow rate	1 mL/min
Isocratic conditions	20 % acetonitrile in water + 0.1 % TFA
Stop time	5 minutes
Injection volume	5 µL, draw speed 100 µL/min, 3 seconds needle wash
Column temperature	40 °C
Detection	273/4 nm, reference wavelength 360/100 nm, 20 Hz

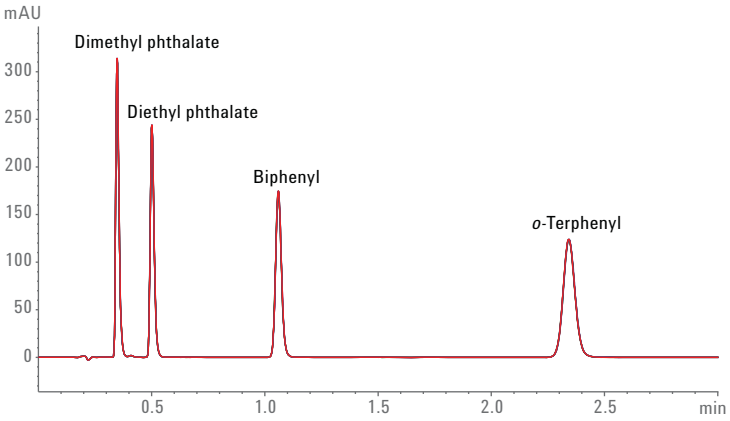
Results and Discussion

Area precision

To evaluate area precision, three different experiments were performed. First, the performance of small injection volumes for 0.5, 1, and 3 µL under isocratic conditions were tested. Figure 1 shows the results. For all injection volumes, the obtained area precisions were excellent, and well below the specification of less than 1 % for 1 to 5 µL.

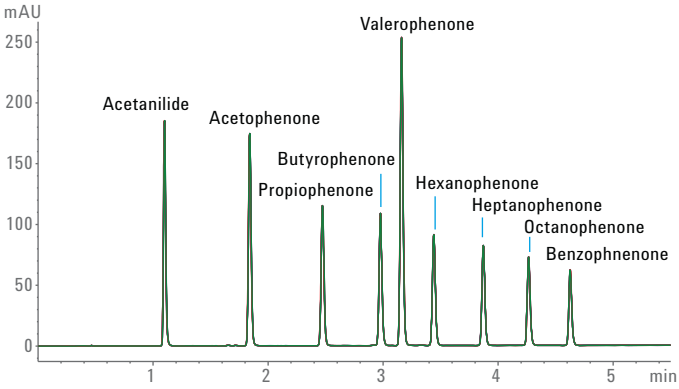
Next, measurements for different injection volumes (1, 3, 5, 10, 15, and 20 µL) applying gradient conditions were conducted. Figure 2 summarizes the results of these experiments.

For all injection volumes, and for all compounds from the RRLC checkout sample, area precisions were within the specification range of less than 1 and 0.25 %, for 1–5 µL and 5–20 µL injection volume, respectively.



Compound	0.5 µL RSD RT (%)	0.5 µL RSD Area (%)	1 µL RSD RT (%)	1 µL RSD Area (%)	3 µL RSD RT (%)	3 µL RSD Area (%)
Dimethyl phthalate	0.02	0.410	0.02	0.279	0.05	0.214
Diethyl phthalate	0.02	0.435	0.02	0.317	0.04	0.266
Biphenyl	0.03	0.423	0.02	0.283	0.03	0.216
o-Terphenyl	0.04	0.399	0.02	0.271	0.03	0.251

Figure 1. Area precision for 0.5, 1, and 3 µL injection volume using isocratic conditions, showing overlay of 10 consecutive runs for 1-µL injection volume, and table of RSD values for retention time (RT) and area.



Compound	1 µL RSD RT	1 µL RSD Area	3 µL RSD RT	3 µL RSD Area	5 µL RSD RT	5 µL RSD Area	10 µL RSD RT	10 µL RSD Area	15 µL RSD RT	15 µL RSD Area	20 µL RSD RT	20 µL RSD Area
Acetanilide	0.07	0.22	0.06	0.23	0.08	0.17	0.05	0.18	0.04	0.12	0.06	0.12
Acetophenone	0.03	0.21	0.03	0.20	0.03	0.13	0.04	0.07	0.03	0.09	0.02	0.13
Propiophenone	0.01	0.24	0.03	0.27	0.02	0.16	0.02	0.08	0.02	0.19	0.02	0.15
Butyrophenone	0.01	0.22	0.01	0.34	0.01	0.17	0.02	0.12	0.02	0.12	0.01	0.19
Valerophenone	0.01	0.30	0.01	0.40	0.01	0.15	0.02	0.17	0.01	0.14	0.02	0.16
Hexanophenone	0.01	0.29	0.02	0.44	0.01	0.13	0.02	0.14	0.01	0.13	0.01	0.23
Heptanophenone	0.01	0.30	0.01	0.40	0.01	0.15	0.02	0.09	0.01	0.14	0.01	0.16
Octanophenone	0.01	0.27	0.01	0.67	0.01	0.24	0.02	0.09	0.01	0.18	0.01	0.20
Benzophenone	0.01	0.36	0.01	0.91	0.01	0.08	0.02	0.07	0.01	0.06	0.01	0.12

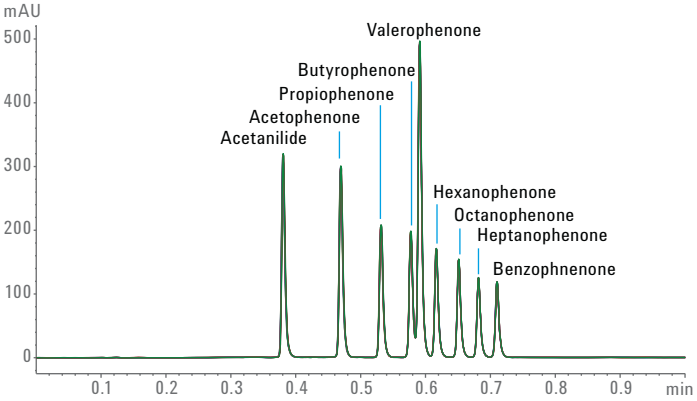
Figure 2. Area precision for several injection volumes applying gradient conditions, showing overlay of 10 consecutive runs for 5-µL injection volume, and a table of RSD values for retention time (RT) and area.

As the last experiment for area precision evaluation, the RRLC checkout sample was analyzed under gradient conditions with a high flow rate of 1.2 mL/min, and low injection volumes of 0.5 and 1 µL. The obtained values are below the specification, and are in good agreement with the results from the previous experiments (Figure 3).

Carryover

To determine carryover, 1,000 ng of chlorhexidine was injected with a 3-second needle wash using 0.1 % TFA, followed by a blank injection. Figure 4 shows the results.

The chromatogram overlay of the chlorhexidine injection and the blank injection clearly demonstrate that no carryover was detected.



Compound	0.5 µL		1 µL	
	RSD RT (%)	RSD Area (%)	RSD RT (%)	RSD Area (%)
Acetanilide	0.07	0.180	0.06	0.217
Acetophenone	0.05	0.183	0.04	0.178
Propiophenone	0.03	0.185	0.03	0.199
Butyrophenone	0.02	0.318	0.02	0.207
Valerophenone	0.02	0.180	0.02	0.161
Hexanophenone	0.02	0.182	0.01	0.152
Heptanophenone	0.01	0.226	0.01	0.159
Octanophenone	0.01	0.228	0.01	0.173
Benzophenone	0.01	0.236	0.01	0.140

Figure 3. Area precision for 0.5 and 1 µL injection volumes using an ultrafast gradient, showing overlay of 10 consecutive runs for 5-µL injection volume, and a table of RSD values for retention time (RT) and area.

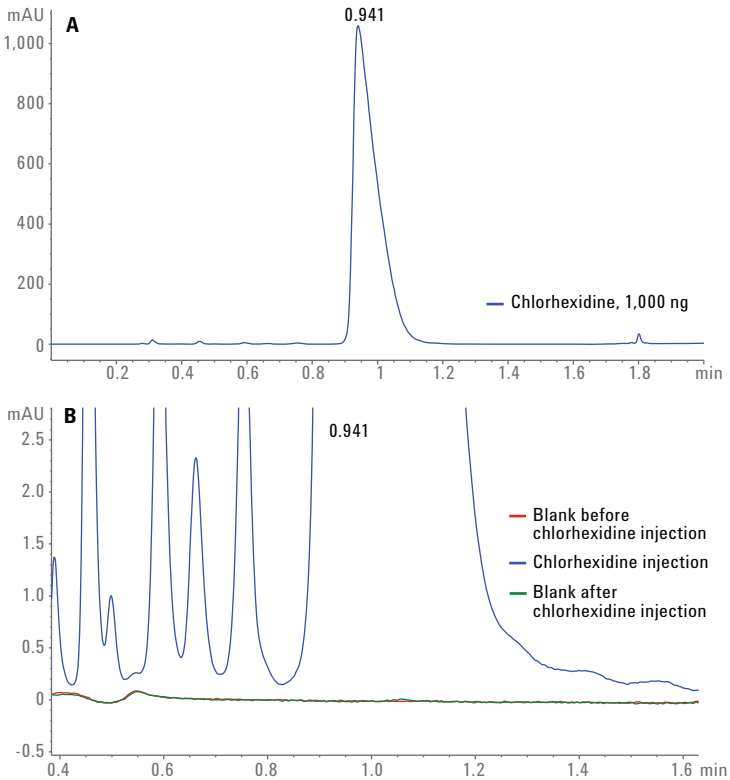


Figure 4. UV chromatogram for carryover evaluation; A) Chlorhexidine analysis, B) Overlay of blank analysis and chlorhexidine injection.

In addition, carryover was analyzed by injecting 2,500 ng of caffeine, followed by a blank injection. The outer needle was washed for 3 seconds with methanol/water (50/50; v/v). Figure 5 shows the chromatograms. Again, no carryover was detected.

Injection volume linearity

For the injection volume linearity experiments, a total amount of 62.5 ng of caffeine was injected in six different concentrations. A stock solution of 125 ng/µL caffeine was diluted five times in 1:2 steps. These resulted in six different injection volumes: 0.5, 1, 2, 4, 8, and 16 µL. For good injection volume linearity, all injections should result in similar peak heights and peak areas for caffeine. Figure 6 shows the obtained results for the analysis. The 1290 Infinity II Vialsampler shows high injection volume linearity for the injection volume range of 0.5 to 16 µL, with an RSD for the area precision and height of below 0.8 %.

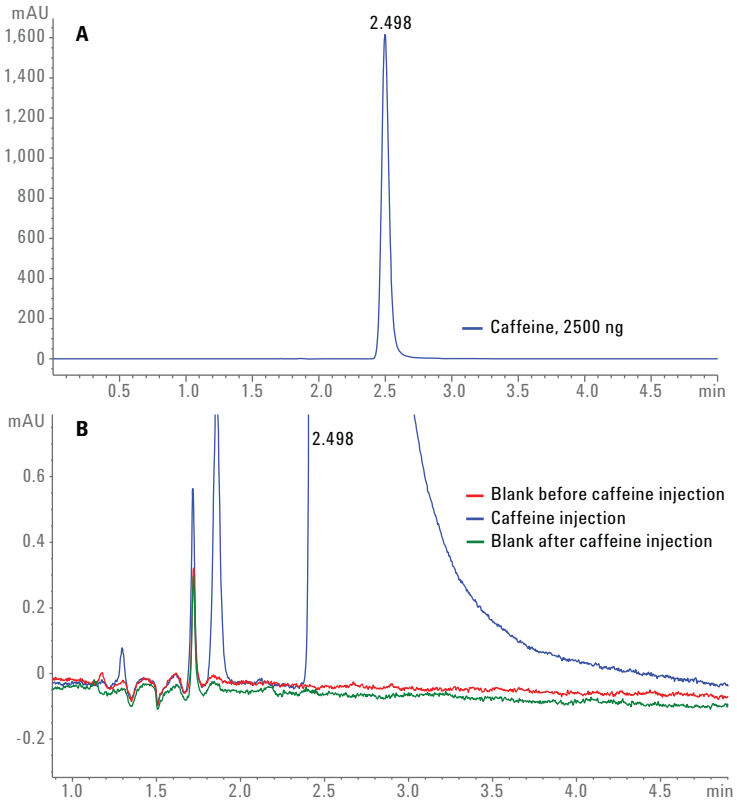
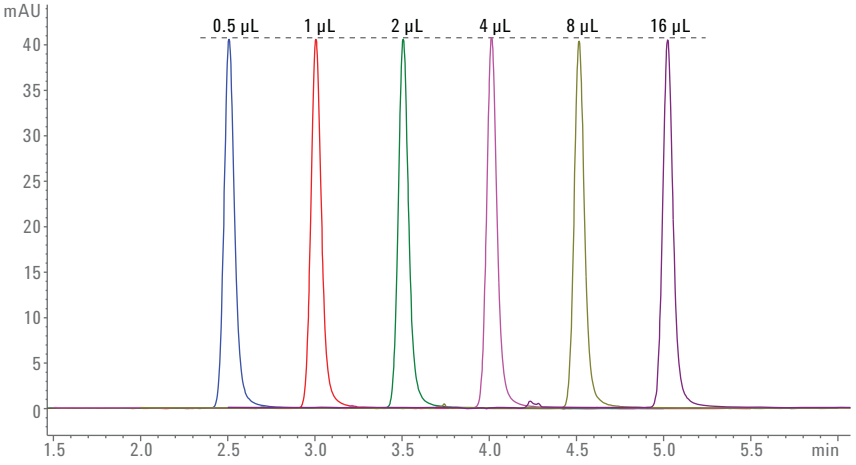


Figure 5. UV chromatogram for carryover evaluation; A) Caffeine analysis, B) Overlay of blank analysis and caffeine injection.



Injection volume (µL)	Area	Height
0.5	175.84	40.57
1	175.30	40.55
2	174.54	40.75
4	174.42	40.90
8	172.22	40.02
16	173.27	40.39
RSD (%)	0.76	0.75

Figure 6. Injection volume linearity measurements using caffeine.

Conclusion

The Agilent 1290 Infinity II Vialsampler shows superior results for the conducted performance tests. The area precision for injection volumes of 5 µL and larger was below 0.25 % RSD, and for smaller injection volumes less than 1 % RSD. Even for ultrafast gradient conditions, the area precisions of the 1290 Infinity II Vialsampler were excellent. The presented carryover experiments for chlorhexidine and caffeine showed no carryover. Furthermore, high injection volume linearity was obtained with an RSD for area and height precision below 0.8 % for injection volumes of 0.5 up to 16 µL. In summary, the 1290 Infinity II Vialsampler offers great performance with high flexibility for the user regarding the optional integration of a column compartment and sample cooler.

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