

Performance Characteristics of the Agilent 1290 Infinity II Multisampler

Technical Overview

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Abstract

This Technical Overview demonstrates the performance of the Agilent 1290 Infinity II Multisampler. Excellent area precision was achieved under isocratic and gradient conditions, under standard conditions as well as with very high pressure and flow rate. High injection volume linearity was attained for injection volumes from 0.78 to 100 μL . In addition, no carryover was detected. The introduction of new design elements – such as the sample hotel, improved handling routines, and the possibility to use different metering devices – provide the user with the highest flexibility.



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Introduction

The Agilent 1290 Infinity II Multisampler is based on the proven flow-through design. Additional elements are the sample hotel for various sample containers – holding up to eight vial plates, the robotics and needle handling routine for shortest injection cycle times, and the multiwash function for lowest carryover. The 1290 Infinity II Multisampler is fully scalable and configurable depending on required application range and sample throughput. It can be equipped optionally with a 100- μ L or 900- μ L metering device, enabling the injection of up to 100 μ L of sample volume at 1,300 bar or 900 μ L at 400 bar.

For the determination of performance characteristics presented in this Technical Overview, the standard configuration of a 1290 Infinity II Multisampler was equipped with an exterior needle wash with one solvent, one needle assembly for injection cycle times of 10 seconds, and one drawer for two vial plates. Performance tests included measurements of area precision at different injection volumes for isocratic and gradient runs using conventional and ultrafast chromatographic conditions. Carryover was determined using UV detection and applying exterior needle wash before the needle was placed into the needle seat. In addition, injection volume linearity was evaluated.

Experimental

Equipment

The Agilent 1290 Infinity LC System consisted of the following modules:

- Agilent 1290 Infinity Diode Array Detector with 10-mm cell (G4212A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity II Multisampler (G7167B) with either standard 40- μ L or 100- μ L metering device (G7167-60043)
- Agilent 1290 Infinity Binary Pump (G4220A)

Solvents and samples

- Agilent RRLC checkout sample (p/n 5188-6529), Set of nine compounds, 100 ng/ μ L each, dissolved in water/ACN (65/35):
 1. Acetanilide
 2. Acetophenone
 3. Propiophenone
 4. Butyrophenone
 5. Benzophenone
 6. Valerophenone
 7. Hexanophenone
 8. Heptanophenone
 9. Octanophenone
- Agilent isocratic standard sample (p/n 01080-68707):
 1. Dimethylphthalate
 2. Diethylphthalate
 3. Biphenyl
 4. *o*-terphenyl
- HPLC-grade acetonitrile, water and chlorhexidine were purchased from Sigma-Aldrich Corp., St. Louis, USA

Isocratic conditions for area precision measurements

Sample	Isocratic standard
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 \times 50 mm, 1.8 μ m (p/n 959757-902)
Mobile phase A	Water:Acetonitrile = 45:55
Flow rate	0.3 mL/min
Column temperature	40 $^{\circ}$ C
Stop time	5 minutes
DAD	254/10 nm, ref. wavelength 380/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	0.5 μ L, draw speed 200 μ L/min, 3 seconds needle wash in flush port
Column	Agilent ZORBAX RRHD SB-C18, 2.1 \times 50 mm, 1.8 μ m (p/n 857700-902)
Mobile phase A	Water:Acetonitrile = 45:55
Flow rate	0.5 mL/min
Column temperature	40 $^{\circ}$ C
Stop time	5 minutes
DAD	254/10 nm, ref. wavelength 380/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	1 and 3 μ L, draw speed 200 μ L/min, 3 seconds needle wash in flush port

Isocratic UHPLC conditions over 1000 bar for area precision measurements

Column	Agilent ZORBAX SB-C18 RRHD, 3 × 50 mm, 1.8 µm (p/n 857700-302)
Mobile phase A	Water:Acetonitrile = 45:55
Flow rate	2.5 mL/min resulting in 1,032 bar
Column temperature	30 °C
Stop time	5 minutes
DAD	254/10 nm, ref. wavelength 380/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	50 µL, draw speed 200 µL/min, 3 seconds needle wash in flush port

Gradient conditions

Sample	Isocratic standard
Column	Agilent ZORBAX Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)
Mobile phase A	Water
Mobile phase B	Acetonitrile
Gradient	20 % B to 95 % B in 5 minutes
Stop time	5.5 minutes
Post time	1 minutes
Flow rate	1.2 mL/min
Column temperature	40 °C
DAD	254/10 nm, ref. wavelength 380/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	5, 10, 15, 20 µL, draw speed 100 µL/min, 3 seconds needle wash in flush port

Ultrafast gradient conditions for area precision measurements

Sample	Agilent RRLC checkout sample
Column	Agilent ZORBAX SB-C18, 2.1 × 50 mm, 1.8 µm (p/n 857700-902)
Mobile phase A	Water
Mobile phase B	Acetonitrile
Flow rate	1.2 mL/min
Gradient	5 % B to 95 % B in 0.5 minutes
Column temperature	60 °C
DAD	254/10 nm, ref. wavelength 380/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	0.5 µL, draw speed 200 µL/min, 3 seconds needle wash in flush port

Conditions for carryover experiments with chlorhexidine

Sample	1,200 ng/µL chlorhexidine, blanks using water
Column	Agilent ZORBAX Eclipse Plus RRHD C18, 2.1 × 50 mm, 1.8 µm (p/n 959757-902)
Mobile phase	Water + 0.1 % TFA:Acetonitrile + 0.1 % TFA, 67:33
Flow rate	0.5 mL/min
Stop time	2.5 minutes
Column temperature	50 °C
DAD	257/4 nm, ref. wavelength 360/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	1 µL, 10 seconds needle wash with methanol:water, 50:50, draw speed 100 µL/min, wait 1.2 seconds

Conditions for carryover experiments with caffeine

Sample	500 µg/mL caffeine, blanks using water
Column	Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
Mobile phase	Water + 0.1 % TFA:Acetonitrile + 0.1 % TFA, 80:20
Flow rate	1 mL/min
Stop time	5 minutes
Column temperature	40 °C
DAD	273/4 nm, ref. wavelength 360/100 nm, 20 Hz, 10-mm path length flow cell
Injection volume	5 µL, 10 seconds needle wash with methanol/water, 50/50, draw speed 100 µL/min, wait 1.2 seconds

Conditions for injection volume linearity measurements

Sample	125 µg/mL caffeine, diluted 5 times at 1:2 for 40-µL metering device; 500 µg/mL caffeine, diluted 7 times at 1:2 for 100-µL metering device
Column	Agilent ZORBAX SB C18, 4.6 × 150 mm, 5 µm (p/n 883975-902)
Mobile phase	Water + 0.1 % TFA:Acetonitrile + 0.1 % TFA, 80:20
Flow rate	1 mL/min
Stop time	5 minutes
Column temperature	40 °C
HDR-DAD	273/4 nm ref. wavelength 360/100 nm, 20 Hz
Injection volume	0.5, 1, 2, 4, 8 and 16 µL, 10 seconds needle wash with methanol:water, 50:50, draw speed 50 µL/min, wait 1.2 seconds

Software

Agilent OpenLAB CDS ChemStation
Edition for LC & LC/MS Systems,
Rev. C.01.06 [61]

Results and Discussion

Design of the Agilent 1290 Infinity II Multisampler

The configuration of the 1290 Infinity II Multisampler used for this performance evaluation offers the following features:

- Internal robotics that move vial plates from the sample hotel to the central workspace for sample injections
- Low carryover to less than 30 ppm using external needle wash
- Injection volumes up to 20 µL with the 40-µL metering device
- Injection volumes up to 100 µL with the 100-µL metering device



Figure 1. Design and interior of the Agilent 1290 Infinity II Multisampler.

The following experiments were performed to determine the precision of areas and carryover:

- Determination of area precision under isocratic conditions by injecting 0.5, 1, and 3 μL with 40- μL and 100- μL metering devices
- Determination of area precision under gradient conditions by injecting 0.25, 0.5, 1, 3, 5, 10, 15, and 20 μL with 40- μL and 100- μL metering devices
- Comparison of area precision for ultrafast conditions using the 1290 Infinity Autosampler and the 1290 Infinity II Multisampler
- Determination of carryover using chlorhexidine and caffeine, external needle wash and UV detection
- Determination of injection volume linearity with 40- μL and 100- μL metering devices

Determination of area precision under isocratic conditions for 0.5, 1, and 3- μL injection volumes

Isocratic conditions were selected to determine the area precision at low injection volumes for the 40- μL metering device as well as for the 100- μL metering device. Typically, small injection volumes are more demanding than larger injection volumes. Figure 2 summarizes the results for injection volumes of 0.5, 1, and 3 μL . Overall the precision was less than 0.14 % RSD for six runs with the 40- μL device and less than 0.3 % RSD for six runs with the 100- μL device.

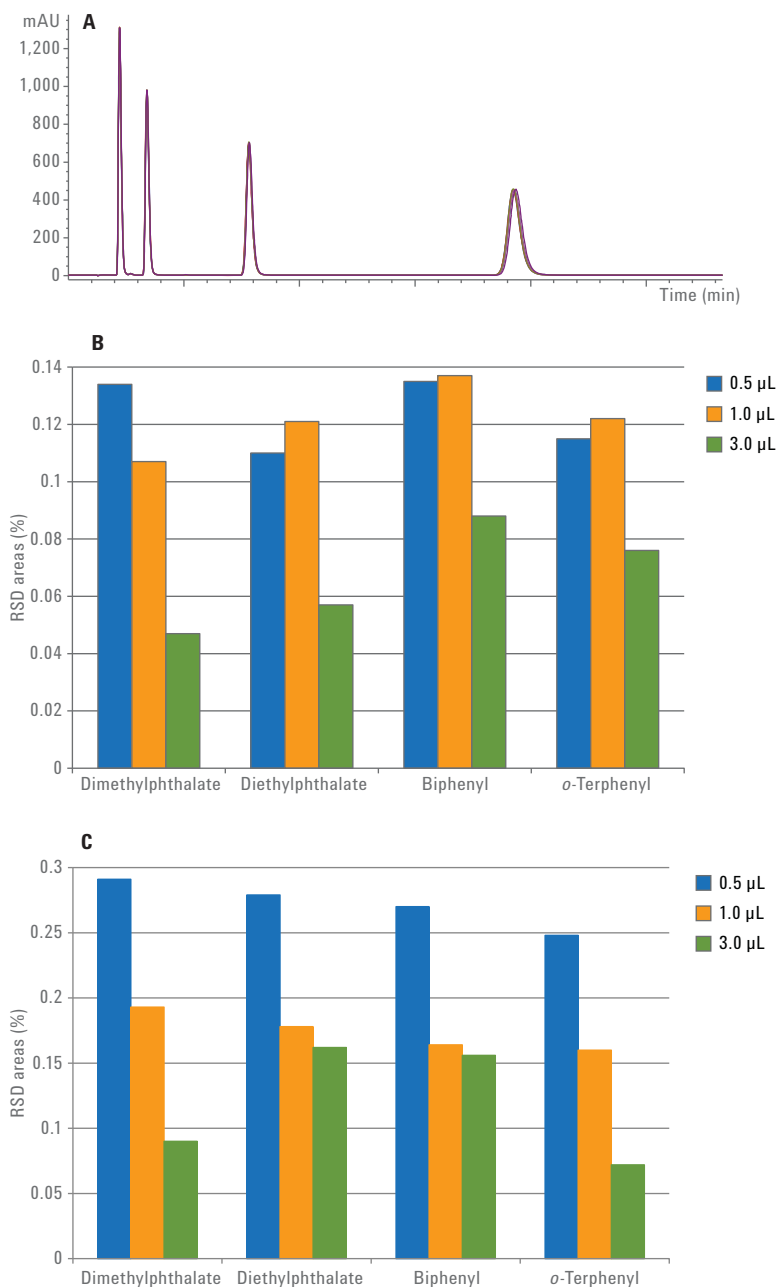


Figure 2. Precision of injection volume at 0.5, 1, and 3 μL applying isocratic conditions using the 40- μL (B) and the 100- μL device (C); the chromatogram (A) shows six overlays.

Determination of area precision under isocratic conditions at ultrahigh pressures over 1,000 bar

Again, isocratic conditions were applied to determine the area precision at ultrahigh pressures over 1,000 bar (2.5 mL/min resulting in 1,032 bar) for injection volumes of 50 μ L with the 100- μ L metering device. Excellent area precisions were achieved with less than 0.061 % RSD for the first two peaks and less than 0.71 % RSD for the last peak, see Figure 3.

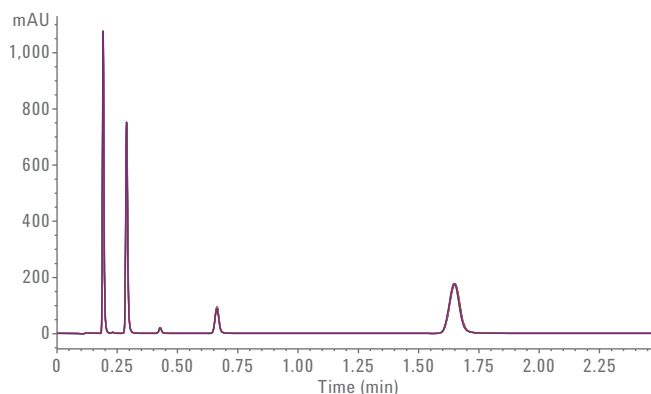


Figure 3. Precision of injection volume of 50 μ L under isocratic conditions at pressures over 1,000 bar using the 100- μ L device, six overlays.

Determination of area precision under gradient conditions for 0.25, 0.5, 1, 3, 5, 10, 15, and 20- μ L injection volumes

Gradient conditions were applied to evaluate the area precision from 0.25- μ L up to 20- μ L injection volume with the 40- μ L metering device, see Figure 4. From 0.5 μ L upwards, the precision was less than 0.14 % RSD. With a precision of less than 1.5 % RSD, the 0.25- μ L injection volume also showed excellent results. As examples, the results for *o*-terphenyl and diethylphthalate are shown.

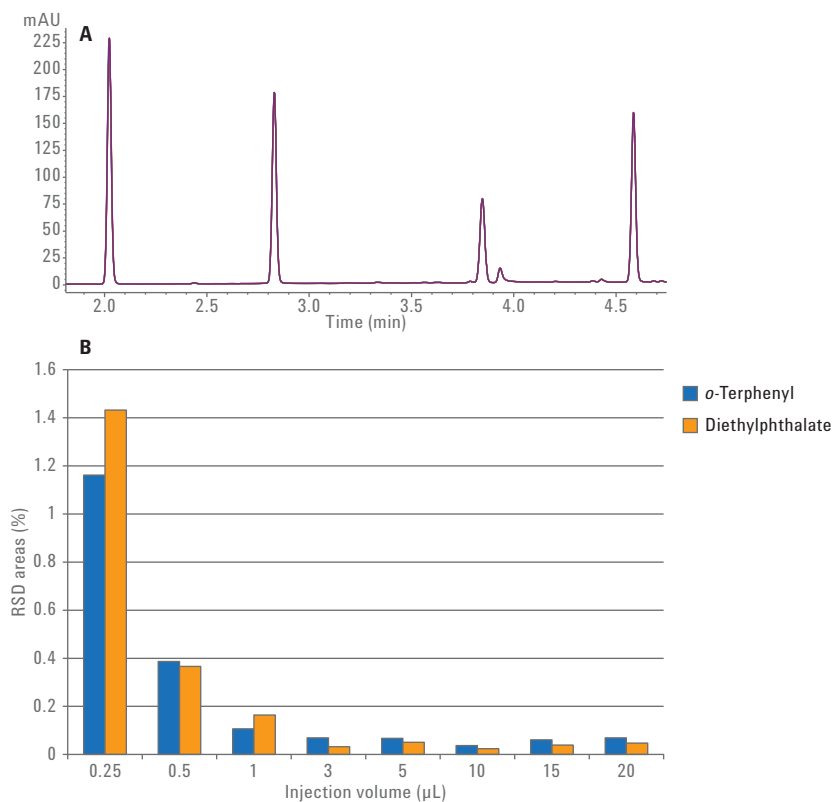


Figure 4. Precision of areas for injection volumes of 0.25 to 20 μ L during a gradient run (injections made using the 40- μ L metering device).

Comparison of area precision under ultrafast conditions using the Agilent 1290 Infinity Autosampler and the Agilent 1290 Infinity II Multisampler

The Agilent 1290 Infinity Autosampler is renowned for achieving precise areas even under demanding conditions. Using a ballistic gradient, high flow rate, and injection volume of 0.5 μL , the area precision was compared with that of the 1290 Infinity II Multisampler. Both autosamplers achieved better results than their respective specifications. Comparing the results, the 1290 Infinity II Multisampler was better than the previous 1290 Infinity Autosampler by a factor of 1.5 to 5.8, see Figure 5.

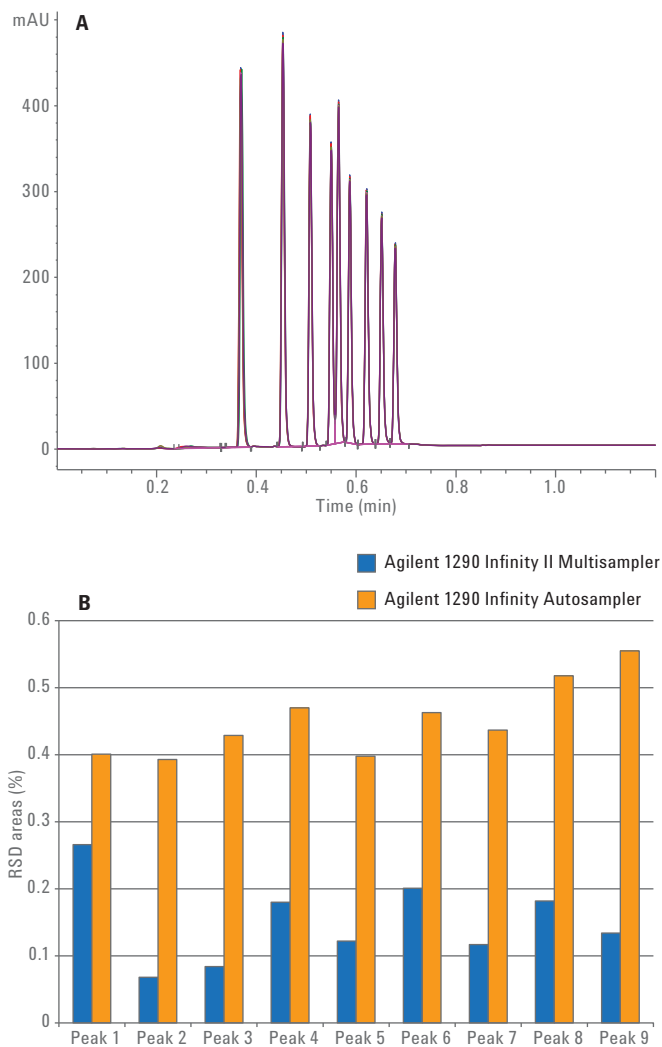


Figure 5. Comparison of area precision of the Agilent 1290 Infinity Autosampler and the Agilent 1290 Infinity II Multisampler for an injection volume of 0.5 μL under ultrafast conditions.

Determination of carryover of chlorhexidine using external needle washing and UV detection

Carryover was measured by injecting 1,200 ng of chlorhexidine, followed by injection of 2 μ L of acetonitrile. Figure 6 shows the chromatograms of the chlorhexidine analysis and the acetonitrile analysis before and after the injection of chlorhexidine. After the injection of chlorhexidine, no carryover was detected for the following injection.

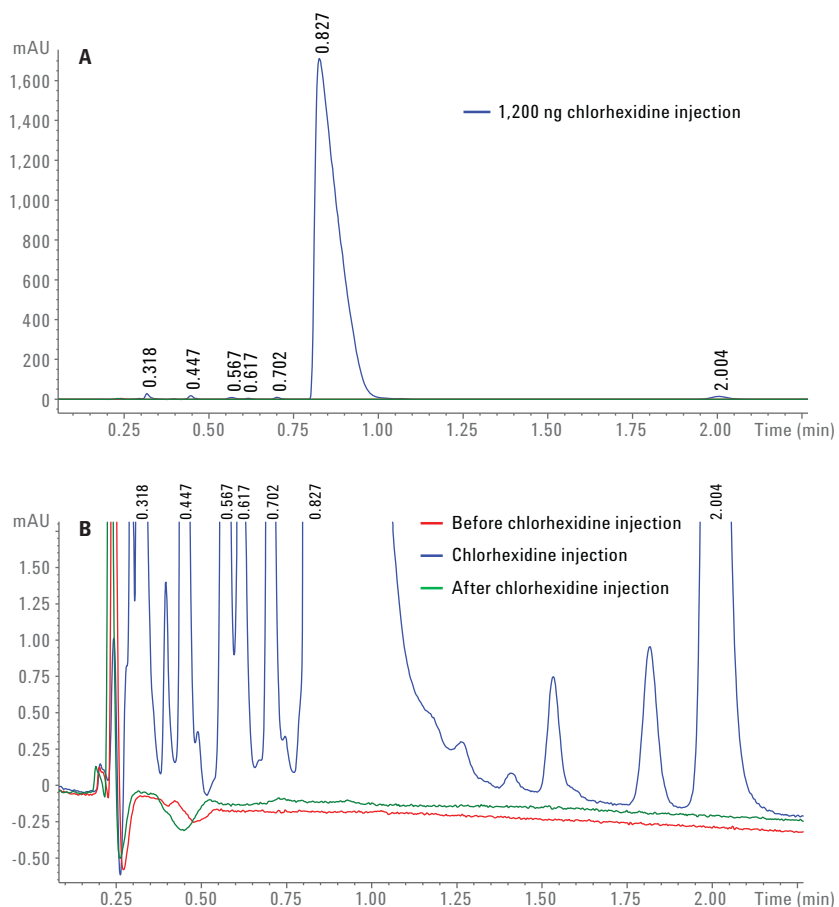


Figure 6. Carryover for the injection of 1,200 ng of chlorhexidine – no carryover was detected.

Determination of carryover of caffeine using external needle washing and UV detection

Carryover was measured by injecting 2,500 ng of caffeine, followed by injection of 5 μ L of water. Figure 7 shows the chromatograms of the caffeine analysis and the water analysis before and after the injection of caffeine.

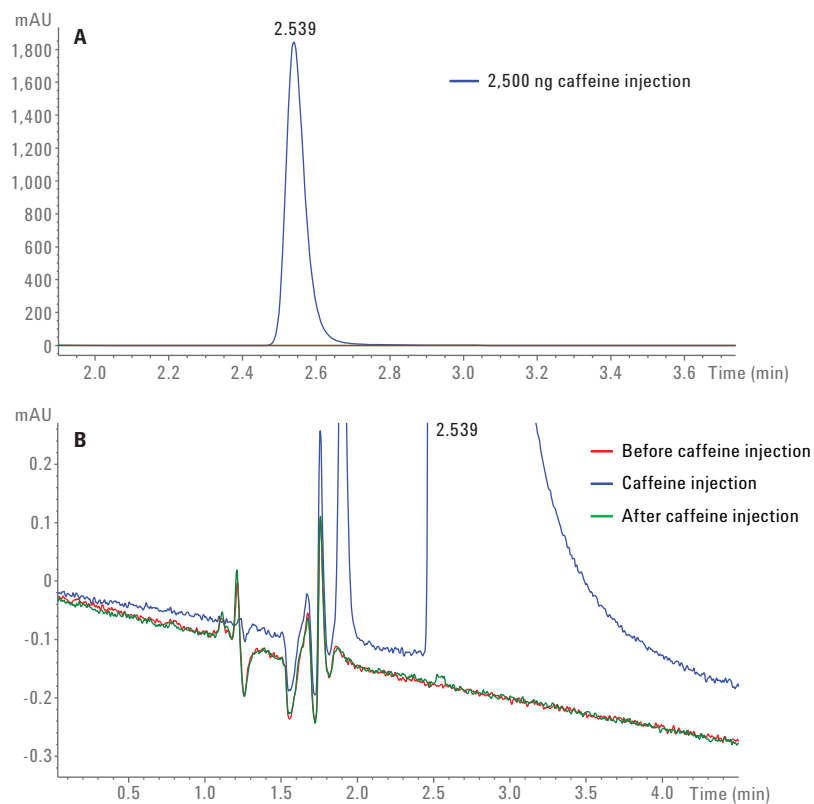


Figure 7. Carryover for the injection of 2,500 ng of caffeine – no carryover was detected.

Determination of injection volume linearity

Injection volume linearity was determined for the 40- μL and 100- μL metering devices. For the 40- μL metering device, a total amount of 62.5 ng of caffeine was injected in six different concentrations (125 $\mu\text{g}/\text{mL}$ of caffeine was diluted five times), resulting in six different injection volumes from 0.5 to 16 μL . For the 100- μL metering device, a total amount of 390 ng of caffeine was injected in eight different concentrations (500 $\mu\text{g}/\text{mL}$ of caffeine was diluted seven times), resulting in eight different injection volumes from 0.78 to 100 μL .

Figure 8 shows overlaid chromatograms of the different injection volumes (panel A for the 40- μL metering device and panel B for the 100- μL metering device). For the 40- μL metering device, the precision of areas over the range from 0.5 to 16- μL injection volume was 1.18 % RSD, and over the range from 1 to 16- μL injection volume 0.322 % RSD. For the 100- μL metering device, the precision was 0.9 % RSD over the complete range from 0.78 to 100- μL injection volume and 0.53 % RSD from 1.56 to 100- μL injection volume.

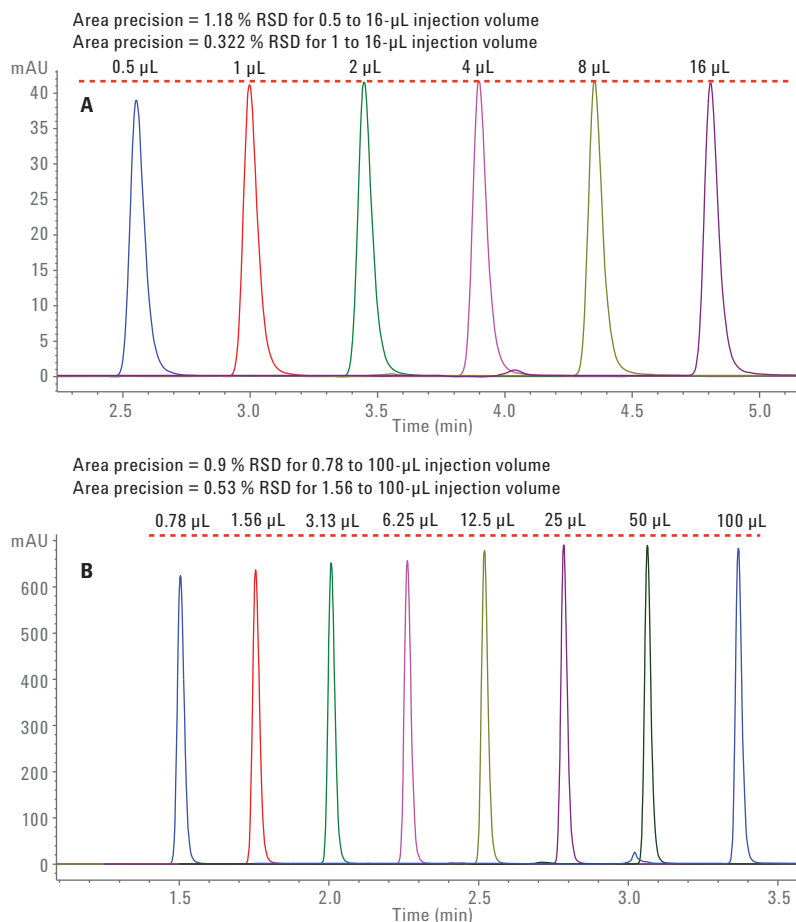


Figure 8. Injection volume linearity for the 40- μL (A) and 100- μL metering device (B).

Conclusion

The Agilent 1290 Infinity II Multisampler exhibited excellent performance in terms of area precision for isocratic and gradient runs with RSDs less than 0.2 % for injection volumes larger than 1 μL . Injection volumes smaller than 1 μL still showed very good results, with RSDs less than 1.5 % for injection volumes down to 0.25 μL . With demanding conditions such as high flow rate and very high pressure above 1,000 bar, very good results were still obtained for area precision. Especially in comparison with the Agilent 1290 Infinity Autosampler – already renowned for superb performance – the 1290 Infinity II Multisampler attained superior results. High injection volume linearity was demonstrated for injection volumes from 0.5 μL to 100 μL (presented for 40- μL and 100- μL metering devices). Moreover, no carryover was detected after injection of high concentrations of chlorhexidine and caffeine. With the introduction of new design elements such as the sample hotel, needle handling routine, and carryover reduction options, the 1290 Infinity II Multisampler offers highest flexibility for sample analysis with highest efficiency.

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