Technical Overview

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Performance Characteristics of the Agilent 1260 Infinity II LC System – an Entry System for Routine Analysis

Authors

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Introduction

The Agilent 1260 Infinity II LC System is the trusted platform that takes you to the next level of routine analysis, giving you the instrument choice needed to achieve the best operational efficiency. This system provides a broad range of reliable instrumentation, which matched with the latest column technologies guarantees robust separation and detection performance. Highest instrument utilization and a fast turnaround cycle are achieved through easy column handling and superior sample logistics from sample submission to data analysis. Designed for method transferability and stepwise upgrade capability, the 1260 Infinity II enables risk-free integration into current infrastructure while matching your budget.

With an operating pressure of up to 600 bar, the 1260 Infinity II quaternary pump is compatible with HPLC and UHPLC. It can handle 2.1, 3, and 4.6 mm id columns over flow rates ranging up to 5 mL/min and semipreparative analysis due to flow rates up to 10 mL/min.

The Agilent 1260 Infinity II vialsampler can optionally house the integrated column compartment for two LC columns with temperature control up to 80 °C and a sample cooler for stable temperatures down to 4 °C, all within one module.

The Agilent 1260 Infinity II variable wavelength detector (VWD) is the most sensitive and fastest detector in its class. Time-programmable wavelength switching provides sensitivity and selectivity for your applications. More sample information can be acquired in the dual-wavelength mode. Low detector noise (<±2.5 µAU) and baseline drift (<1 × 10^{-4} AU/h) facilitate precise quantification of trace levels components. High productivity can be achieved with fast analysis at up to 120 Hz data rates.

Experimental

Equipment

The Agilent 1260 Infinity II Quaternary System used for the experiments consisted of the following modules:

- Agilent 1260 Infinity II quaternary pump (G7111B)
- Agilent 1260 Infinity II vialsampler (G7129A) with integrated column compartment (G7130A), 100 µL loop, and 100 µL metering device
- Agilent 1260 Infinity II variable wavelength detector (G7114B) with 10 mm standard cell

Software

Agilent OpenLab CDS ChemStation Edition Rev. 2.4

Columns

- Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (part number 695975-902T)
- Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm (part number 699975-302)

Chemicals

All solvents were LC grade. Acetonitrile and methanol were purchased from Merck (Darmstadt, Germany). Fresh ultrapure water was obtained from a Milli-Q Ultrapure lab water system equipped with a Millipak 0.22 µm membrane point-of-use cartridge (Millipore, Merck (Darmstadt, Germany)). Formic acid was purchased from VWR (Darmstadt, Germany). TFA was purchased from Merck (Darmstadt, Germany).

Samples

The Agilent Enterprise Edition Caffeine extended standards kit (part number 5190-0579) contains caffeine standards of nine different concentration levels.

The Agilent isocratic standard sample (part number 01080-68707) consisted of four compounds:

- Dimethyl phthalate
- Diethyl phthalate
- Biphenyl
- *o*-Terphenyl

The Agilent RRLC checkout sample (part number 5188-6529) consisted of nine compounds:

- Acetanilide
- Acetophenone
- Propiophenone
- Butyrophenone
- Valerophenone
- Hexanophenone
- Heptanophenone
- Octanophenone
- Benzophenone

The Agilent LC/MS seven analyte system suitability standard (part number 5191-4544) consisted of seven compounds:

- Amitriptyline hydrochloride
- Diethyl phthalate
- Diamyl phthalate
- Dihexyl phthalate
- Dioctyl phthalate
- 8-Bromoguanosine hydrate
- 4-Chlorocinnamic acid

A Reversed Phase test mix (Order No. 47641-U) was ordered from Sigma Aldrich and consisted of four compounds:

- Uracil
- Phenol
- *n,n*-Diethyl-*m*-toluamide
- Toluene

HPLC gradient system diagnostic mix (Order No. 48271) was ordered from Sigma Aldrich and consisted of seven compounds:

- Phenol
- Methyl paraben
- Ethyl paraben
- Propyl paraben
- Butyl paraben
- Heptylparaben
- Uracil

Chlorhexidine and caffeine were purchased from Sigma-Aldrich.

Methods

Pump performance

Table 1. Chromatographic parameters for retention time (RT) precision measurements under isocratic conditions.

Parameter	Value
Sample	Isocratic sample
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A: Water B: Acetonitrile
Gradient	50% B Stop time: 7 min
Injection	Injection volume: 5 µL Draw speed 100 µL/min, 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.5 mL/min
Temperature	40 °C
Detection	UV
UV	254 nm, 10 Hz

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

Parameter	Value
Sample	Reversed phase test mix and HPLC gradient system diagnostic mix, 1 mL of each diluted to 5 mL with Water/ACN (1:1) and mixed 1:1
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	0 min – 30% B 15 min – 85% B Stop time: 15 min Post time: 5 min
Injection	Injection volume: 5 µL 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.0 mL/min
Temperature	30 °C
Detection	UV
UV	254 nm, 10 Hz

Table 3. Chromatographic parameters for RT precision measurements under fast gradient conditions.

Parameter	Value
Sample	RRLC checkout sample
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	0 min – 30% B 1.5 min – 95% B Stop time: 2 min Post time: 1 min
Injection	Injection volume: 1 µL 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	2.0 mL/min
Temperature	50 °C
Detection	UV
UV	245 nm, 40 Hz

Injector performance

Table 4. Chromatographic parameters for injection volume linearity experiments.

Parameter	Value
Sample	Caffeine 125 ng/µL, five times diluted 1:2 dilution
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water +0.1% TFA B) Acetonitrile +0.1% TFA
Gradient	20% B Stop time: 3 min
Injection	Injection volume: 0.5 / 1 / 2 / 4 / 8 / 16 µL Draw Speed 100 µL/min, 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.0 mL/min
Temperature	40 °C
Detection	UV
UV	273 nm, 20 Hz

Table 5. Chromatographic parameters for area precision measurements under isocratic conditions.

Parameter	Value
Sample	Isocratic sample
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	50% B Stop time: 7 min
Injection	Injection volume: 1 / 3 / 5 µL Draw Speed 100 µL/min, 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.5 mL/min
Temperature	40 °C
Detection	UV
UV	254 nm, 40 Hz

Table 6. Chromatographic parameters for area precision measurements under gradient conditions.

Parameter	Value
Sample	RRLC checkout sample
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	0 min – 20% B 11 min – 95% B Stop time: 11.5 min Post time: 1 min
Injection	Injection volume: 1 / 3 / 5 / 10 / 20 µL Draw Speed 100 µL/min, 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.2 mL/min
Temperature	40 °C
Detection	UV
UV	254 nm, 20 Hz

Table 7. Chromatographic parameters for carryover experiments using chlorhexidine.

Parameter	Value
Sample	Chlorhexidine 1 mg/mL
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A) Water +0.1% TFA B) Acetonitrile +0.1% TFA
Gradient	33% B Stop time: 4.5 min
Injection	Injection volume: 1 µL Draw Speed 100 µL/min, 3 s needle wash with water +0.1% TFA
Flow rate	1.5 mL/min
Temperature	50 °C
Detection	UV
UV	257 nm, 20 Hz

Table 8. Chromatographic parameters for carryover experiments using caffeine.

Parameter	Value
Sample	Caffeine 500 ng/µL
Column	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm
Solvent	A) Water +0.1% TFA B) Acetonitrile +0.1% TFA
Gradient	20% B Stop time: 3 min
Injection	Injection volume: 5 µL Draw Speed 100 µL/min, 3 s needle wash with water/methanol (50/50; v/v)
Flow rate	1.0 mL/min
Temperature	40 °C
Detection	UV
UV	273 nm, 20 Hz

Detector performance

Table 9. Chromatographic parameters for detector linearity measurements.

Parameter	Value
Sample	Enterprise edition caffeine extended standards kit
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A) Water B) Acetonitrile
Gradient	10% B Stop time: 3.5 min
Injection	Injection volume: 1 µL 10 s needle wash with water/methanol (50/50; v/v)
Flow rate	0.6 mL/min
Temperature	30 °C
Detection	UV
UV	273 nm, 20 Hz

LC/MS seven analyte mix

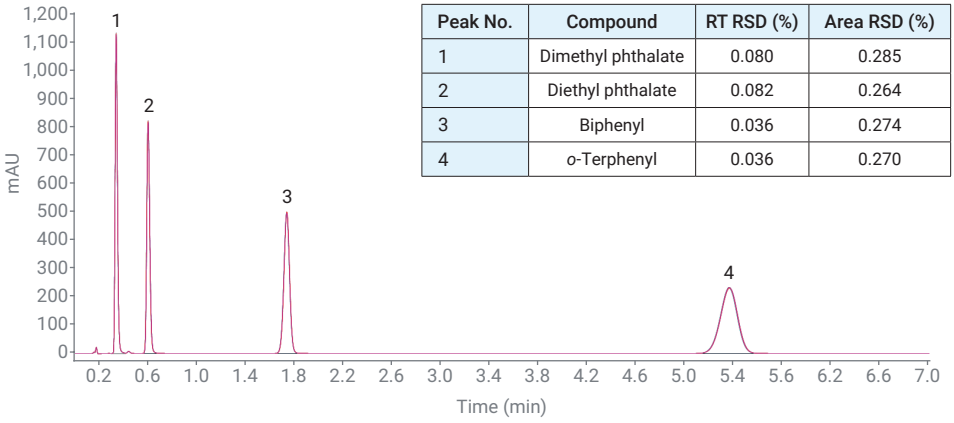
Table 10. Chromatographic parameters for area and RT precision measurements using an LC/MS seven analyte system suitability standard.

Parameter	Value
Sample	LC/MS seven analyte system suitability standard
Column	Agilent InfinityLab Poroshell 120 EC-C18, 3 × 50 mm, 2.7 µm
Solvent	A) Water +0.1% FA B) Acetonitrile +0.1% FA
Gradient	0 min – 2%B 2 min – 100%B Stop time: 4 min Post time: 2 min
Injection	Injection volume: 0.5 µL
Flow rate	1.0 mL/min
Temperature	45 °C
Detection	UV
UV	254 nm, 20 Hz

Results and discussion

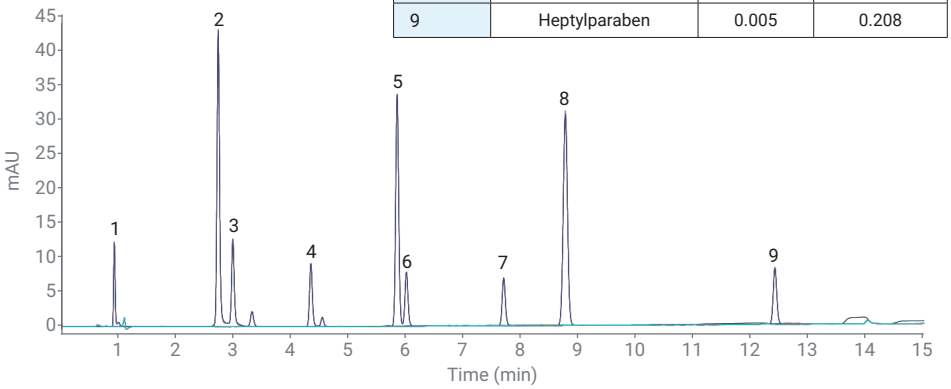
Pump performance: retention time precision

The most important parameter influencing retention time (RT) precision, is pump performance. RT precision was tested with different gradients and isocratic conditions using 4.6 and 2.1 mm id columns. The relative standard deviation (RSD) of RTs for isocratic conditions was <0.09% RSD for n = 7 (Figure 1). Figure 2 shows conventional gradient runs with an RT precision of <0.03% RSD for n = 7. For fast gradients, with a run time of approximately 2 minutes, the RT precision was <0.11% RSD for n = 7 (Figure 3).



Peak No.	Compound	RT RSD (%)	Area RSD (%)
1	Dimethyl phthalate	0.080	0.285
2	Diethyl phthalate	0.082	0.264
3	Biphenyl	0.036	0.274
4	*o*-Terphenyl	0.036	0.270

Figure 1. RT precision for isocratic conditions, showing an overlay of seven consecutive runs, and a table of RSD values for retention time (RT) and area.



Peak No.	Compound	RT RSD (%)	Area RSD (%)
1	Uracil	0.028	0.201
2	Phenol	0.014	0.136
3	Methylparaben	0.019	0.356
4	Ethylparaben	0.020	0.246
5	*n,n*-Diethyl-*m*-toluamide	0.011	0.114
6	Propylparaben	0.007	0.163
7	Butylparaben	0.009	0.125
8	Toluene	0.007	0.627
9	Heptylparaben	0.005	0.208

Figure 2. Retention time (RT) precision for conventional gradient runs, showing an overlay of seven consecutive runs (blue line = blank), and a table of RSD values for RT and area.

Injector performance: injection volume linearity

For injection volume linearity experiments, a total 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 dilutions resulted in six different injection volumes: 0.5, 1, 2, 4, 8, and 16 µL. For high injection volume linearity, all injections should result in similar peak heights and peak areas for caffeine. Figure 4 shows the obtained results for the analysis. The 1260 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%.

Peak No.	Compound	RT RSD (%)	Area RSD (%)
1	Acetophenone	0.051	0.543
2	Propiophenone	0.104	0.398
3	Butyrophenone	0.069	0.475
4	Valerophenone	0.016	0.361
5	Hexanophenone	0.017	0.538
6	Heptanophenone	0.017	0.532
7	Octanophenone	0.029	0.451
8	Benzophenone	0.052	0.568
9	Acetanilide	0.051	0.521

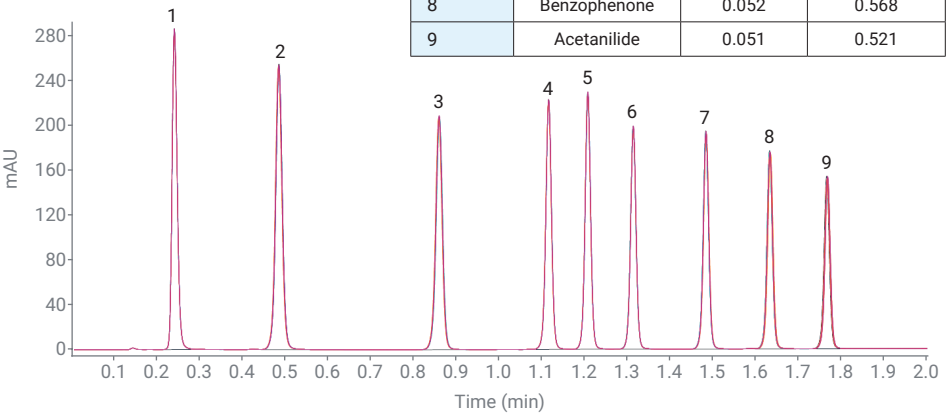
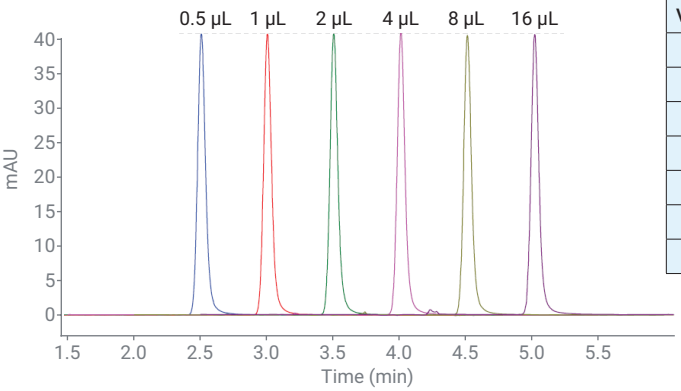


Figure 3. Retention time precision for fast gradient runs, showing an overlay of seven consecutive runs, and a table of RSD values for RT and area.



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 4. Injection volume linearity measurements using caffeine.

Injector performance: area precision

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

Next, measurements for different injection volumes (1, 3, 5, 10, and 20 µL) applying gradient conditions were conducted. Figure 6 summarizes the results of these experiments. For all injection volumes, and for all compounds of the RRLC checkout sample, area precisions were within the specification range of less than 1% for 1 to 5 µL and less than 0.25% for injection volume >5 µL.

Peak No.	Compound	1 µL		3 µL		5 µL	
		RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD (%)
1	Dimethyl phthalate	0.088	0.4601	0.056	0.3303	0.080	0.2850
2	Diethyl phthalate	0.078	0.4269	0.060	0.2936	0.082	0.2642
3	Biphenyl	0.041	0.4657	0.059	0.3124	0.036	0.2749
4	*o*-Terphenyl	0.023	0.4917	0.034	0.3086	0.036	0.2702

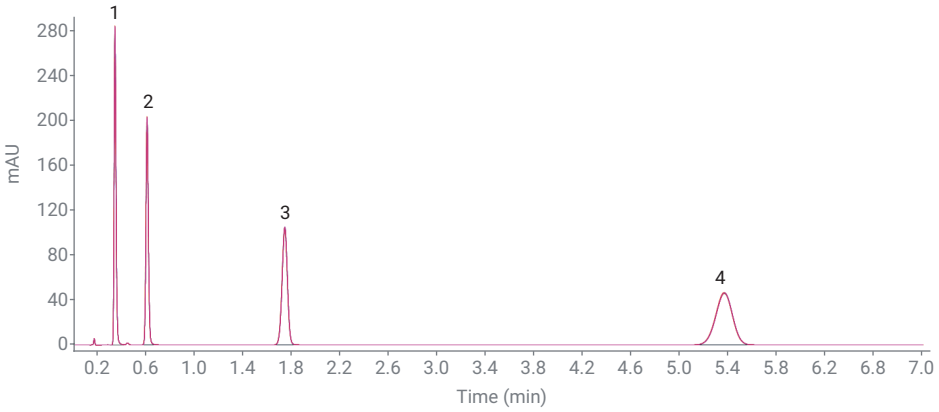


Figure 5. Area precision for 1, 3, and 5 µL injection volume using isocratic conditions, showing an overlay of seven consecutive runs for 1 µL injection volume, and a table of RSD values for retention time (RT) and area.

Peak No.	Compound	1 µL		3 µL		5 µL		10 µL		20 µL	
		RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD (%)	RT RSD (%)	Area RSD(%)	RT RSD (%)	Area RSD (%)
1	Acetanilide	0.313	0.484	0.044	0.104	0.042	0.095	0.354	0.057	0.063	0.052
2	Acetophenone	0.088	0.428	0.019	0.096	0.027	0.116	0.124	0.151	0.024	0.058
3	Propiophenone	0.034	0.400	0.013	0.102	0.016	0.110	0.063	0.129	0.017	0.068
4	Butyrophenone	0.019	0.420	0.019	0.127	0.013	0.129	0.040	0.149	0.014	0.072
5	Valerophenone	0.018	0.443	0.018	0.085	0.016	0.088	0.036	0.083	0.010	0.046
6	Hexanophenone	0.022	0.365	0.017	0.102	0.012	0.146	0.033	0.141	0.009	0.097
7	Heptanophenone	0.013	0.322	0.010	0.112	0.009	0.133	0.025	0.193	0.007	0.085
8	Octanophenone	0.010	0.284	0.009	0.110	0.008	0.144	0.025	0.129	0.009	0.063
9	Benzophenone	0.013	0.198	0.010	0.131	0.009	0.164	0.024	0.163	0.009	0.082

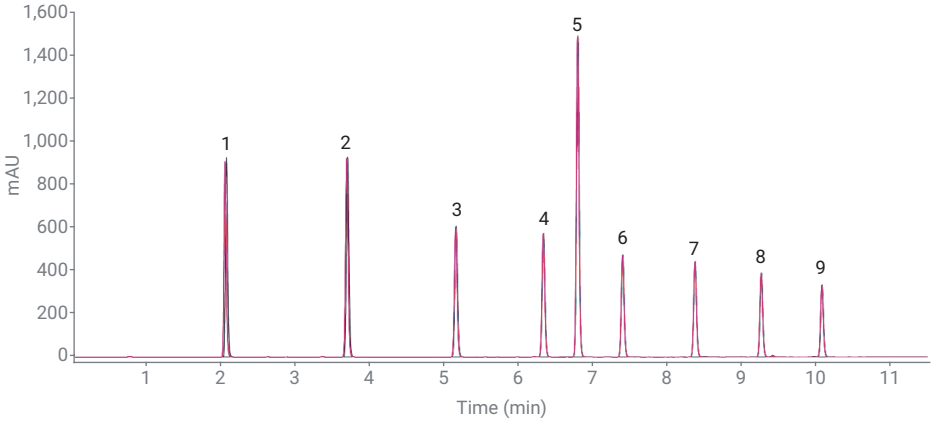


Figure 6. Area precision for 1, 3, 5, 10, and 20 µL injection volume using conventional gradient runs, showing an overlay of seven consecutive runs for 10 µL injection volume, and a table of RSD values for retention time (RT) and area.

Injector performance: carryover

To determine the carryover, 1,000 ng of chlorhexidine was injected with a 3-second needle wash using 0.1% TFA, followed by a blank injection. Figure 7 shows a chromatogram overlay of the chlorhexidine injection and the blank injection, clearly demonstrating that no carryover was detected.

Carryover was also analyzed by injecting 2,500 ng of caffeine, followed by a blank injection. The needle was washed for 3 seconds with methanol/water (50/50 v/v). Figure 8 shows the chromatograms. Again, no carryover was detected.

Detector performance: linearity

Linearity was tested using caffeine standards from 1.5 to 800 ng injected with n = 7. Good linearity was obtained at this concentration range. The response factors were within the 5% error range over an absorbance range of 1.7 to 1,085 mAU (Figure 9).

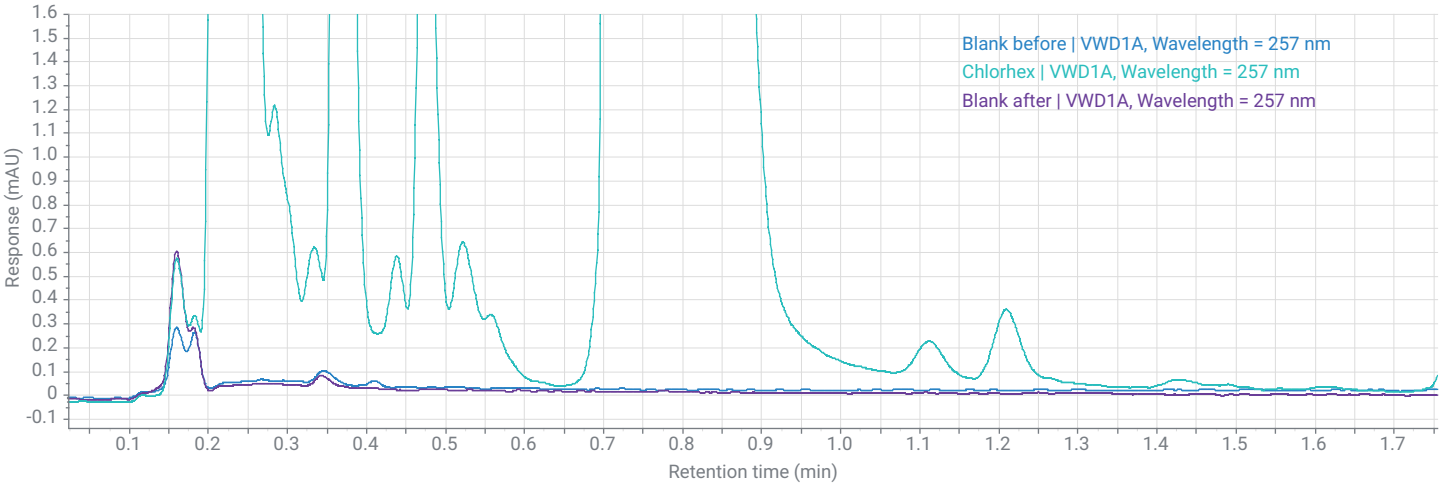


Figure 7. UV chromatogram for carry over evaluation. Overlay of chlorhexidine injection (green) and blank analysis before (blue) and after chlorhexidine injection (purple).

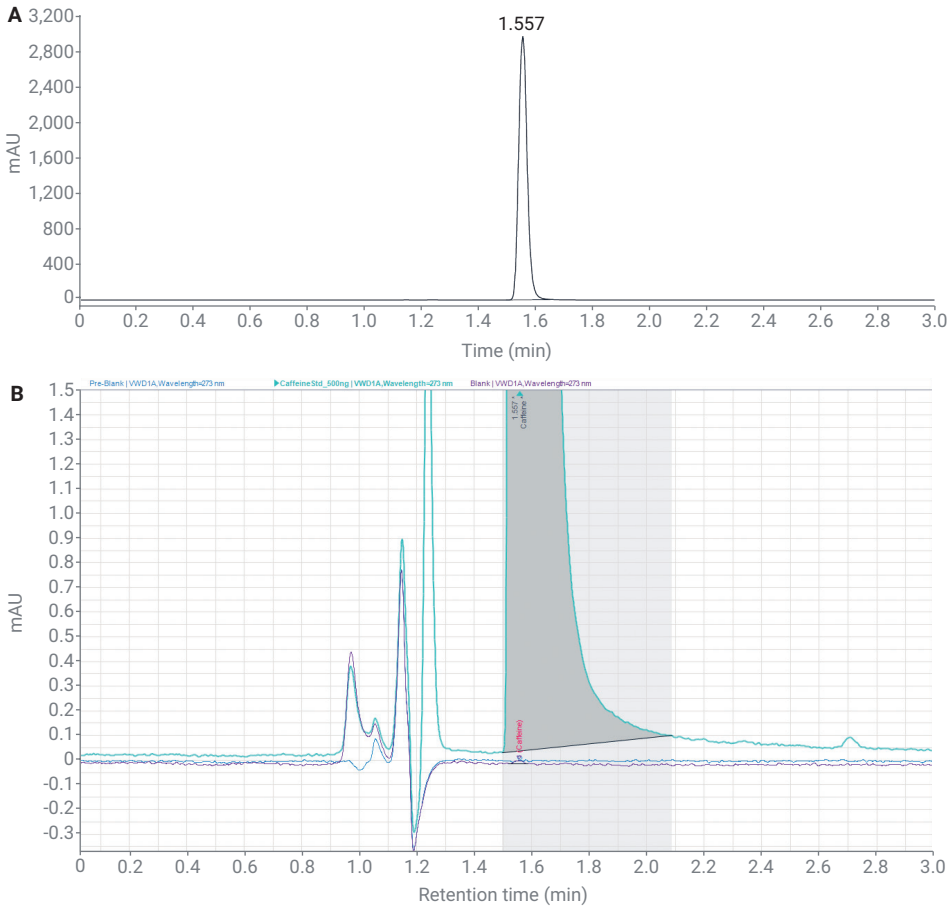


Figure 8. UV chromatogram for carry over evaluation; A) Caffeine analysis, B) Overlay of caffeine injection (green) and blank analysis before (blue) and after caffeine injection (purple)

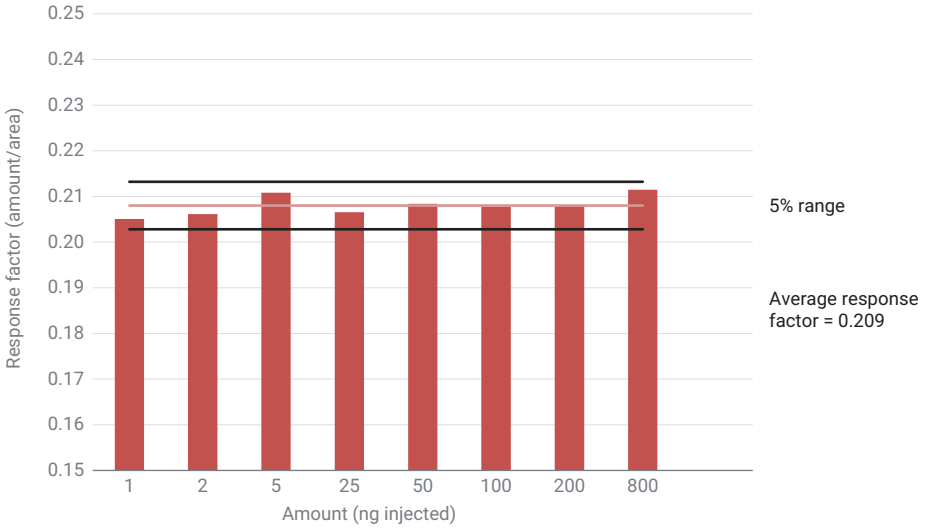


Figure 9. Linearity of the Agilent 1260 Infinity II VWD with standard flow cell.

LC/MS seven analyte system suitability standard: application

In addition to the performance tests presented previously, a LC/MS seven analyte system suitability standard was used to test measurement performance. The analysis showed outstanding results for RT precision and good results for area precision, with an injection volume of 0.5 µL. Figure 10 shows the results.

Conclusion

The performance of the Agilent 1260 Infinity II Quaternary System meets the requirements of modern analytical liquid chromatography. The precision of retention times for conventional LC is typically <0.04% RSD. The precision for peak areas is typically <0.25% for injection volumes >5 µL, and for smaller injection volumes less than 1% RSD. The presented carry over experiments for 1,000 ng chlorhexidine and 2,500 ng caffeine injections showed no carry over. Furthermore, high injection volume linearity was obtained with an RSD for area and height precision below 0.5% for injection volumes between 0.5 and 16 µL.

The Agilent 1260 Infinity II VWD showed good linearity results.

Peak No.	Compound	RT RSD (%)	Area RSD (%)
1	8BG	0.029	1.488
2	Amitriptyline	0.030	1.505
3	4CCA	0.021	2.056
4	DEP	0.028	0.997
5	DAP	0.040	0.919
6	DHP	0.040	1.453
7	DOP	0.042	0.773

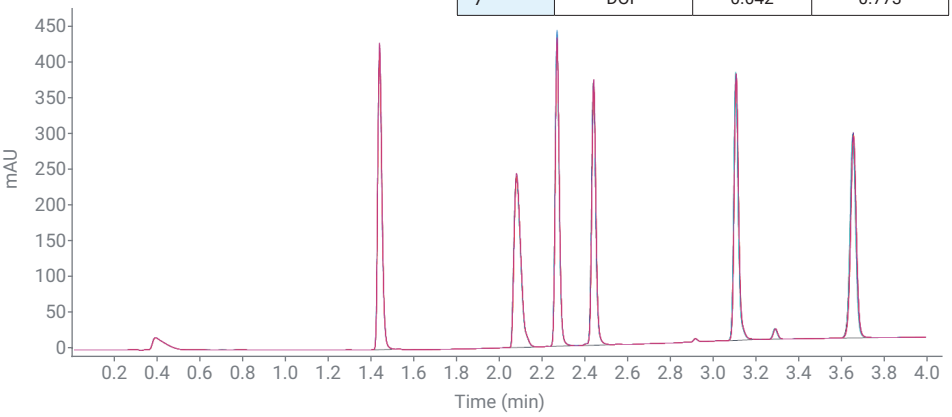


Figure 10. Area and retention time (RT) precision for 0.5 µL injection volume using conventional gradient runs, showing overlay of seven consecutive runs, and a table of RSD values for retention time (RT) and area.

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