Performance characteristics of the Agilent 1290 Infinity Autosampler

Highest precision for areas for conventional and ultrafast LC with lowest carryover and fast cycle times

Technical Note

Introduction

A main performance criterion of any LC system is area precision, which is influenced primarily by the autosampler. The Agilent 1290 Infinity Autosampler is based on the flow-through or variable loop design. An injection valve switches the sample flow path (seat, injection needle, loop and metering device) in and out of the pump flow path. The sample flow path is switched out of the pump flow path when the sample is drawn. Then the injection valve is switched and the sample is injected onto the column by the flow provided by the pump. The sample flow path typically stays in the pump flow path during the complete run time and is therefore continuously flushed. The advantage of this type of injector is that precise injections of both large and small volumes are possible without the necessity of hardware changes. In addition, no sample is wasted since the drawn sample volume is injected.

In this Technical Note the area precision for fast gradient and isocratic applications is evaluated, and the minimum accessible volume and experiments for providing lowest carryover are performed.

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The new design

The design of the Agilent 1290 Infnity Autosampler is based on the next generation of flow-through autosampler with a reduced hydraulic volume of 80 µL. This design offers:

- High precision of small and large injection volumes without hardware changes from 0.1 to 20 µL (40 µL optional)

- No sample losses

- Overlapped injection and automatic delay volume reduction for higher speed

- New cartridge loop design for easy exchange of injection loop

- Peltier temperature control from 4 to 40 °C for thermally-labile samples (requires an Agilent 1200 Series Thermostat)

Equipment and material

The instrument used was an Agilent 1290 Infinity LC system, equipped with the following modules:

- Agilent 1290 Infinity Binary Pump

- Agilent 1290 Infinity Autosampler

- Agilent 1290 Infinity Thermostatted Column Compartment

- Agilent 1290 Infinity Diode Array Detector for 160-Hz operation

- Agilent ZORBAX Rapid Resolution High Definition (RRHD) SB C-18 columns with different internal diameters and lengths, packed with 1.8-µm particle
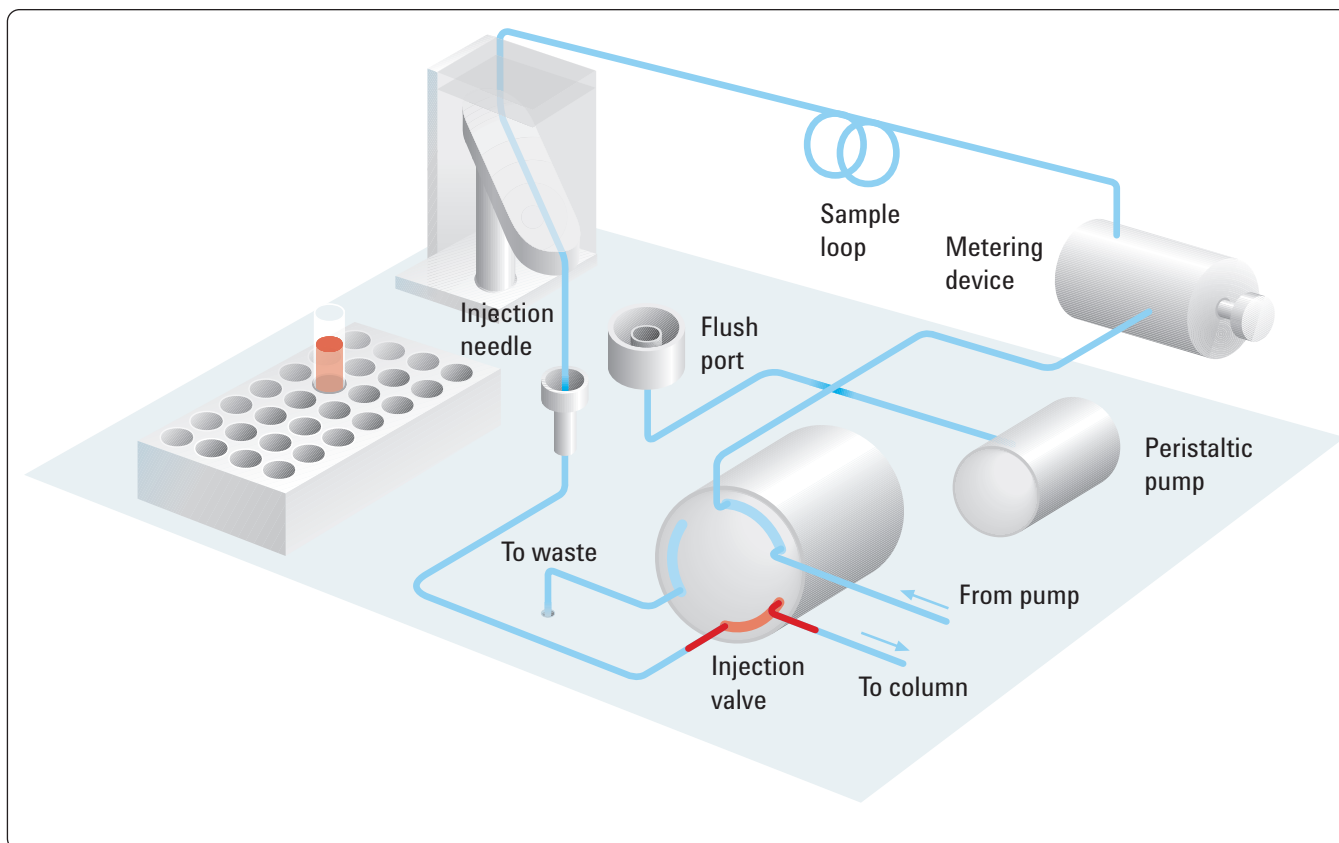

Figure 1
Design of Agilent 1290 Infinity Autosampler.

Area precision for 1-µL and 3-µL injection volumes for conventional isocratic runs

Precise injection is mandatory for good quantitative results in liquid chromatography (LC). The Agilent 1290 Infinity Autosampler can inject precisely over an injection volume range of 0.1 µL to 20 µL, or optionally up to 40 µL. An example of the precision of 1-µL and 3-µL injections using conventional conditions is shown here (Figure 2). The precision was <0.161% relative standard deviation (RSD) overall. The results are summarized here (Table 1).

Peak	RSD(%) 1 µL	RSD(%) 3 µL
1	0.0985	0.0714
2	0.0856	0.114
3	0.1074	0.1600
4	0.115	0.1397

Table 1
Precision of areas using conventional isocratic conditions.

Area precision for 0.5 µL at ultrafast gradient conditions

Figure 3 shows the area precision for an ultrafast gradient application. All 9 peaks eluted within 1 minute. The area precision was determined for injection volumes of 0.5 µL. The precision was below 0.56% RSD.

The precision data for an injection volume of 0.5 µL at ultrafast conditions are combined and presented here (Table 2).

Peak	RSD Area 0.5 uL
1	0.401
2	0.393
3	0.429
4	0.470
5	0.398
6	0.463
7	0.437
8	0.518
9	0.555

Table 2
Precision for an injection volume of 0.5 µL at ultrafast gradient runs.

Chromatographic conditions:

Sample:	Agilent isocratic standard sample (p/n 01080-68707)
Column:	Agilent ZORBAX SB-C18, 2.1 mm × 50 mm
Mobile phase:	Mixed out of 2 channels 45/55% water/acetonitrile
Flow rate:	0.5 mL/minutes
Column temperature:	40 °C
Detector:	245/10 nm, ref 380/100 nm, 20 Hz, slit 4 nm
Injection volume:	1 µL and 3 µL
Stop time:	5.5 minutes
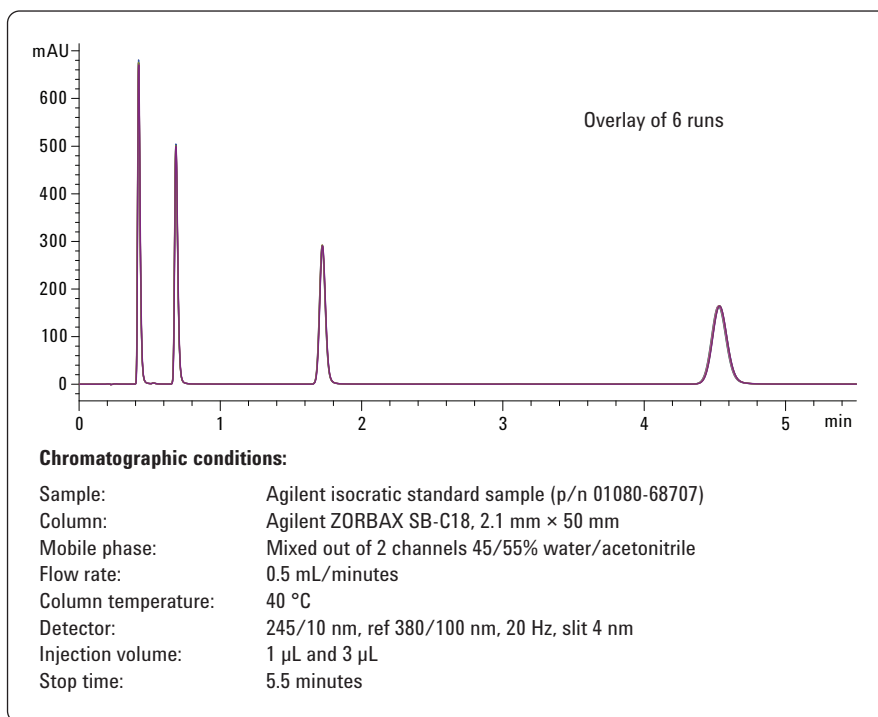
Figure 2
Precision of areas for 1 and 3 µL injections.

Chromatographic conditions:

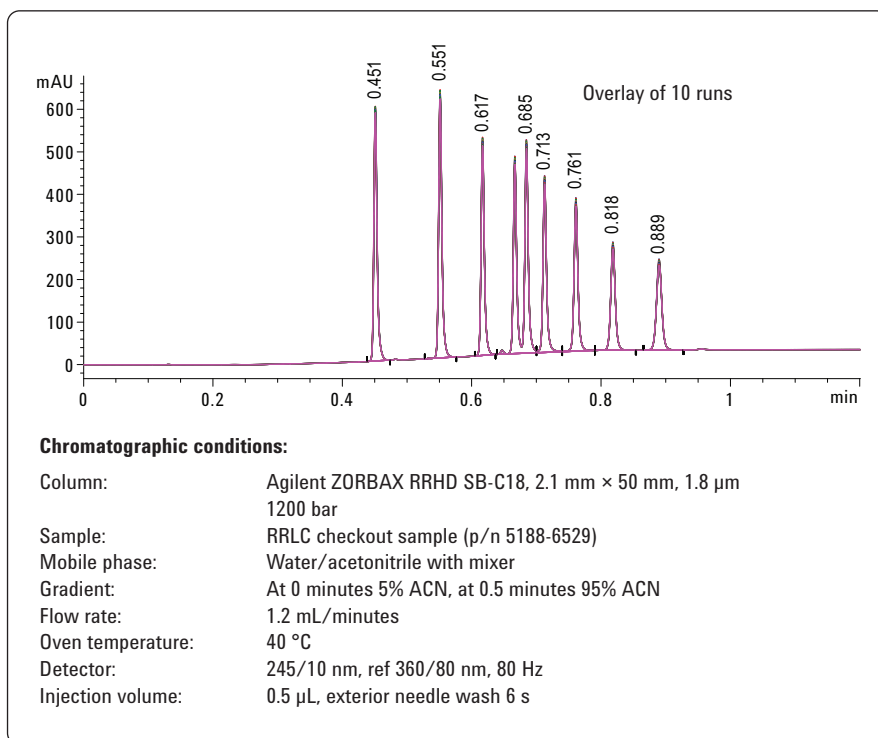|	
Column:	Agilent ZORBAX RRHD SB-C18, 2.1 mm × 50 mm, 1.8 µm 1200 bar
Sample:	RRLC checkout sample (p/n 5188-6529)
Mobile phase:	Water/acetonitrile with mixer
Gradient:	At 0 minutes 5% ACN, at 0.5 minutes 95% ACN
Flow rate:	1.2 mL/minutes
Oven temperature:	40 °C
Detector:	245/10 nm, ref 360/80 nm, 80 Hz
Injection volume:	0.5 µL, exterior needle wash 6 s

Figure 3
Analysis at ultrafast conditions and an injection volume of 0.5 µL.

Carryover

Carryover was tested using the Agilent 1290 Infinity Autosampler with exterior needle wash for 10 seconds (Figure 4). The carryover was found to be <0.003% for the conditions used. After a 1200-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. If the wash bottle is empty, the flush port not primed, or the flush port not cleaned correctly, the injector must be cleaned by backflushing the seat, seat capillary and valve groove.

Backflush of seat and seat capillary

Using ChemStation, lift the needle up and disconnect the capillary coming from the pump at the injection valve. Connect the capillary to port 4 of the injection valve. Start pumping with 5 mL and flush for about 2 minutes. The instrument is now ready to restore method conditions and begin analysis.

Minimum accessible sample volume

Another important parameter is the minimum accessible volume, which strongly depends on the sample vial used. Small vials with conical bores and volumes as low as 100 µL are best
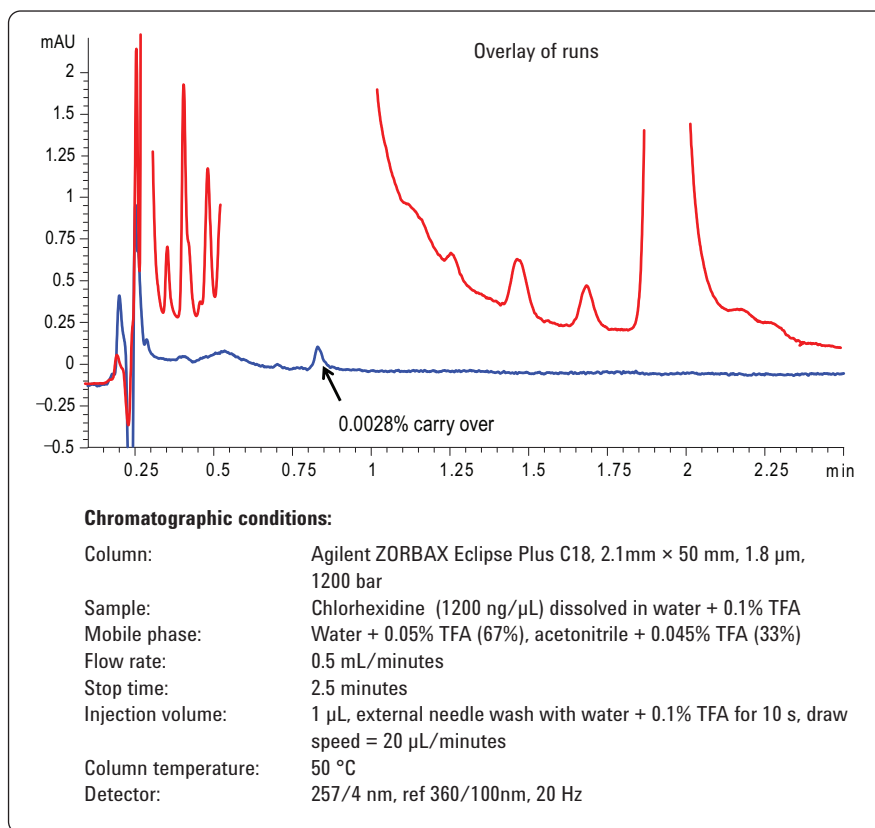

Chromatographic conditions:

Column:	Agilent ZORBAX Eclipse Plus C18, 2.1mm × 50 mm, 1.8 µm, 1200 bar
Sample:	Chlorhexidine (1200 ng/µL) dissolved in water + 0.1% TFA
Mobile phase:	Water + 0.05% TFA (67%), acetonitrile + 0.045% TFA (33%)
Flow rate:	0.5 mL/minutes
Stop time:	2.5 minutes
Injection volume:	1 µL, external needle wash with water + 0.1% TFA for 10 s, draw speed = 20 µL/minutes
Column temperature:	50 °C
Detector:	257/4 nm, ref 360/100nm, 20 Hz

Figure 4
Carryover less than 0.003% for chlorhexidine.

suited to access even 1 µL out of a 3-µL sample volume. In order to draw 1 µL out of 3 µL, it is necessary to activate the "well/vial bottom sensing" feature in the autosampler setup screen.

Conclusion

The Agilent 1290 Infinity Autosampler provides excellent precision over an injection volume range from 0.1 up to 20 µL and optionally to 40 µL. The minimum accessible sample amount is 1 µL extracted from 3 µL using a conical 100-µL vial. The carryover is due to the flow-through design and the external needle wash routine, typically < 0.004% for chlorhexidine.

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