Proof of Performance

Enhancing the maximum injection volume of the Agilent 1290 Infinity Autosampler

Technical Overview

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Abstract

This Technical Overview demonstrates the performance of the Agilent 1290 Infinity Autosampler with increased injection volume capability of up to 120 µL at the full power range. Data is presented showing the retention time stability, the linearity, and the area precision of injections with injection volumes from 20 µL to 120 µL using the 1290 large volume injection kit.

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Introduction

The standard configuration of the Agilent 1290 Infinity Autosampler has a maximum injection volume of 20 µL. An injection volume of up to 40 µL is possible using the 40-µL flex loop kit (p/n 5067-4703). With the 1290 large volume injection kit (G4216A), the maximum injection volume can be increased to 120 µL at full power range with only an additional seat capillary. Injecting such high volumes is of high interest whenever sample enrichment is necessary for the detection of low level trace compounds in diluted samples without the need for major hardware changes.

This Technical Overview demonstrates the performance of the Agilent 1290 Infinity Autosampler with increased injection volume capability up to 120 µL. Data is presented showing the retention time stability, the linearity, and the area precision of injections with volumes from 20 µL to 120 µL using the 1290 large volume injection kit.

Experimental

Equipment

Agilent 1290 Infinity LC System consisting of:

- Agilent 1290 Infinity Binary Pump

- Agilent 1290 Infinity Thermostatted Column Compartment

- Agilent 1290 Infinity Diode Array Detector (DAD)

- Agilent 1290 Infinity Autosampler equipped with a 40 µL loop and the 1290 large volume 1200 bar injection kit

Column

Agilent ZORBAX Eclipse Plus RRHD, 2.1 × 50 mm, 1.8 µm

Software

Agilent OpenLAB ChemStation Edition revision C.01.03

HPLC method

Agilent 1290 Infinity Binary Pump

Solvent A: water
Solvent B: acetonitrile
Gradient: 0 min – 10% B, 1.0 min – 10% B, 1.1 min – 55% B
Stop time: 10 min
Post time: 3 min

Agilent 1290 Infinity Autosampler

Equipped with the 40 µL flex loop kit (p/n 5067-4703) and 1290 large volume injection kit (G4216A), which contains a capillary mounted between needle seat and injection valve.

Injection volumes: 20, 40, 60, 80, 100 and 120 µL (10 replicates each).

Needle wash: 6 s in methanol
Draw speed: 200 µL/min
Eject speed: 100 µL/min

Advanced delay
volume reduction: On, with a flush out factor of 5

- The delay volume reduction function was used to ensure retention time identity over all injection volumes used. The advance delay volume reduction function can also be deselected without compromising the retention time precision. In case of compounds prone to carryover, the flush-out factor can be enhanced up to 20, the enrichment step in the beginning of the separation has to be prolonged accordingly. In addition, the valve cleaning capability of the autosampler can be used to remove sticky compounds.

Agilent 1290 Infinity Thermostatted Column Compartment

Column temperature: 50 °C

Agilent 1290 Infinity Diode array Detector

Data acquisition: 254/4 nm; Ref. 360/100 nm; slit 4 nm
Data rate: 20 Hz
Cell: 10 mm Max-Light

Sample

LC isocratic sample (p/n 01080-68704)

Content

Compound 1:   dimethyl phthalate (1.19 g/L),
Compound 2:   diethyl phthalate (1.19 g/L),
Compound 3:   biphenyl (0.08 g/L),
Compound 4:   o-terphenyl (0.24 g/L).

Diluted 1:100 in 10 % acetonitile/water for use.

Results and discussion

The Agilent 1290 Infinity Autosampler is able to inject up to 20 µL of sample[1] in the standard configuration. With the 40-µL flex loop kit, this can be increased to 40 µL[2]. Larger volumes at full pressure rating without major hardware change can be injected by using the 1290 large volume injection kit with an additional capillary mounted between the injection needle seat and the injection valve in the autosampler. When selecting the large injection volume option in the user interface of OpenLab ChemStation C01.03 the autosampler performs an automated multi-draw procedure where several draw cycles of 20 or 40 µL are alternated with ejection to the extended seat capillary. The final injection is performed by switching the complete specified injection volume into the mainpass of the autosampler.

As a test, the LC isocratic sample was injected in an Agilent 1290 Infinity LC System with an autosampler equipped with the 1290 large volume injection kit. 20, 40, 60, 80, 100, and 120 µL injections with 10 replicates per injection volume were applied. The overlay of the chromatograms shows increasing peak areas and constant retention times (Figure 1).

The retention time performance was determined by calculating the relative standard deviation for each compound and injection volume (Table 1). Table 1 shows that the retention time precision for all compounds is between 0.03% and 0.1%.

The linearity is displayed in the calibration curves for each compound using the peak areas of each injection volume (Figure 2). All correlation coefficients are higher than 0.99982 and the correlation for Compounds 1 and 2 is higher than 0.99993. The relative standard deviation of the area precision for Compounds 1 and 2 is 0.060% and
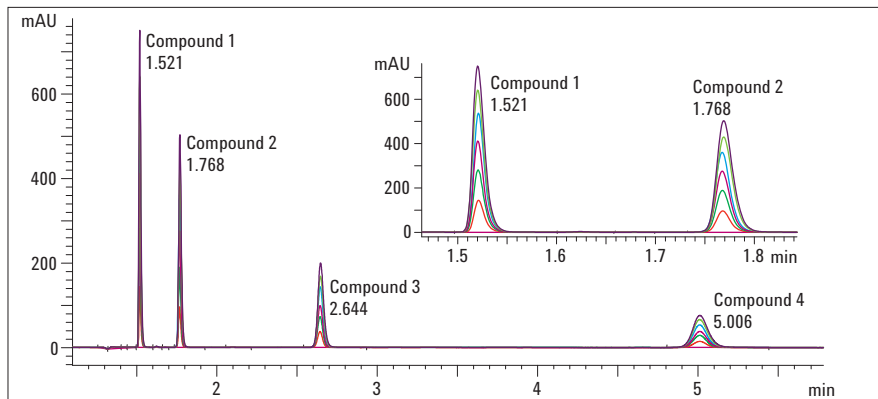

Figure 1)
Overlay of 6 injection volumes, 20, 40, 60, 80, 100, and 120 µL. Compound 1: dimethyl phthalate, Compound 2: diethyl phthalate, Compound 3: biphenyl, Compound 4: o-terphenyl.

	Compound 1 retention time		Compound 2 retention time		Compound 3 retention time		Compound 4 retention time	
	mean	RSD [%]	mean	RSD [%]	mean	RSD [%]	mean	RSD [%]
20 µL	1.521	0.036	1.768	0.029	2.643	0.052	5.011	0.099
40 µL	1.521	0.026	1.768	0.042	2.641	0.095	5.002	0.126
60 µL	1.521	0.027	1.768	0.042	2.642	0.092	5.003	0.126
80 µL	1.522	0.036	1.767	0.025	2.643	0.031	5.007	0.039
100 µL	1.517	0.029	1.764	0.064	2.626	0.084	4.955	0.119
120 µL	1.518	0.029	1.763	0.031	2.626	0.061	4.951	0.125
Mean	**1.520**	**0.031**	**1.766**	**0.039**	**2.637**	**0.069**	**4.988**	**0.106**

Table 1
Retention time precision for each compound at the used injection volumes.
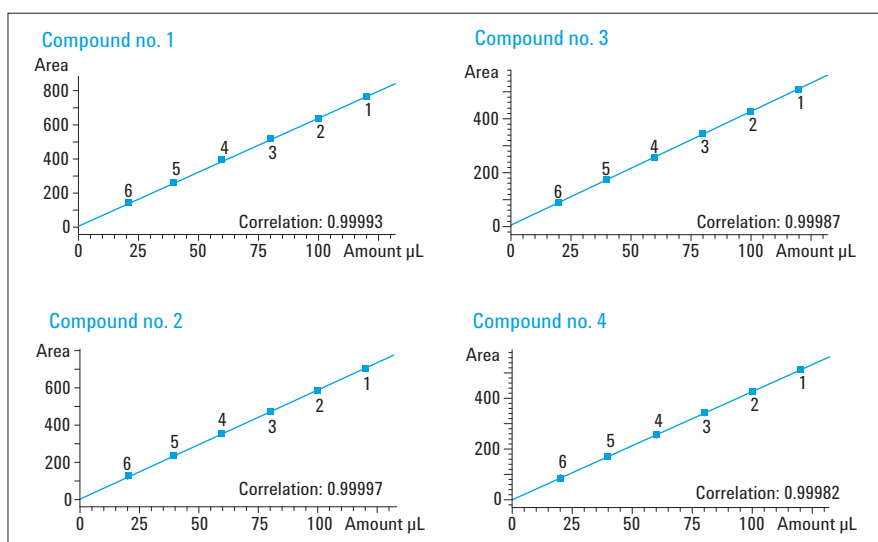

Figure 2
Linearity of injection volumes 20, 40, 60, 80, 100, 120 µL.
Correlation Compound 1: 0.99993
Correlation Compound 2: 0.99997
Correlation Compound 3: 0.99987
Correlation Compound 4: 0.99982

0.089%, respectively (Table 2). The relative standard deviation of the area precision of Compounds 3 and 4 has a broader distribution due to the broader late eluting peaks but is typically below 0.25%.

Conclusion

This Technical Overview demonstrates the use of the 1290 large volume injection kit for injection volumes up to 120 µL at full power range with the Agilent 1290 Infinity Autosampler. The relative standard deviation of the retention times is typically better than 0.1%, linearity correlation factors are between 0.99982 and 0.99997. The relative standard deviation of the area precision is typically below 0.25%. The results prove the "proof of performance" of the 1290 large volume injection kit in the 1290 Infinity Autosampler without major hardware change at full pressure rating for analytical workflows whenever sample enrichment is necessary.

	Compound 1 area RSD [%]	Compound 2 area RSD [%]
20 µL	0.121	0.131
40 µL	0.063	0.061
60 µL	0.063	0.061
80 µL	0.051	0.083
100 µL	0.026	0.118
120 µL	0.033	0.079
Mean	**0.060**	**0.089**

Table 2
Area precision for Compounds 1 and 2 at the used injection volumes.

References

1.
Agilent Technical Note "Performance characteristics of the Agilent 1290 Infinity Autosampler", Agilent Publication Number 5990-5292EN, **2010**.

2.
Agilent Technical Note "Performance characteristics of the 40 µL injection loop of the Agilent 1290 Infinity Autosampler", Agilent Publication Number 5990-5421EN, **2010**.

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