

Injecting large sample volumes with the Agilent 1100 Series using the multi-draw and 900-µl injection upgrade kits

Technical Note

Abstract

The standard and thermostatted Agilent 1100 Series autosamplers offer the possibility to inject volumes from 0.1 µl to 100 µl with high precision. However, for certain applications, such as semi-preparative HPLC or low concentrated samples, higher sample volumes are required.

This technical note describes the enhanced injection volume capabilities, which are possible with the multi-draw upgrade kit, the 900-µl injection upgrade kit, a combination of both kits, and a 5000-µl seat capillary.





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Introduction

The standard and thermostatted Agilent 1100 Series autosamplers offer the possibility to inject volumes from 0.1 µl to 100 µl with high precision¹. However, for certain applications, such as semipreparative HPLC or low concentrated samples, higher sample volumes are required.

In semi-preparative HPLC more sample amount is applied to the column. This can be achieved by concentration or volume overloading. Concentration overloading is only possible if the solubility of the sample compound in the liquid phase is high enough. Otherwise volume overloading with injection volumes in the milliliter range is necessary.

Samples with low compound amounts have to be concentrated for proper detection. This can be done offline by evaporating the solvent or online by accumulating the compound on the head of the column using an appropriate eluent. After the sample is completely applied to the column the eluent is changed and separation and detection can be achieved. Another possibility is to concentrate the compound on the head of a small column and then flush back the compound onto another column where the actual separation takes place. This requires a column-switching valve, which can be built into the Agilent 1100 Series thermosatted column compartment.

Agilent Technologies offers two upgrade kits to enhance the injection volume:

- multi-draw upgrade kit, and
- 900-µl injection upgrade kit.

They can be used independently or in combination with each other. Larger injection volumes can be achieved using other seat capillaries of larger volume.

Equipment

All experiments were carried out on the Agilent 1100 Series high pressure gradient system consisting of

- an Agilent 1100 Series vacuum degasser,
- an Agilent 1100 Series binary pump,
- an Agilent 1100 Series autosampler,
- an Agilent 1100 Series thermostatted column compartment, and
- an Agilent 1100 Series diode array detector.

The system was controlled using the Agilent ChemStation software (rev. A.06.03).

The multi-draw upgrade kit, consisting of a 400-µl and a 1400-µl extended seat capillary was installed according to the *Installation Note for Multidraw Upgrade Kit²* which is supplied with the product.

The 900-µl injection upgrade kit, consisting of a 900-µl analytical head, a 900-µl loop extension and a 900-µl needle was installed according to the *Installation Note* for 900-µl Injection Upgrade Kit³ which is supplied with the product.

Configurations

Various configurations of sample loop and seat capillary are possible as listed in table 1. Configuration examples 1-5 are discussed in this technical note.

The configuration of the sampling system is shown in figure 1.

Figure 2 shows the sample loop and the seat capillary configurations in the *Injector Configuration* window of the ChemStation. Here the sample loop is called *Syringe*.

Configuration	Sample loop (µl)	Seat capillary (µl)	Max. injection volume	900-µl injection upgrade kit	Multidraw upgrade kit
Standard	100 µl	2.3 µl	100 µl (standard)	no	no
Example 1	100 µl	400 µl	500 µl	no	yes
Example 2	100 µl	1400 µl	1500 µl	no	yes
Example 3	900 µl	2.3 µl	900 µl	yes	no
Example 4	900 µl	1400 µl	1800 µl (2300 µl*)	yes	yes
Example 5	900 µl	5000 µl**	5900 µl	yes	no

* Using an injector program

** Seat capillary not included in the multi-draw upgrade kit

Table 1

Overview of configurations



Figure 1 Schematic of the Agilent 1100 Series autosampler

Injector Configuration : Instrument 1	x		
Volumes	external Contacts		
<u>Syringe:</u> μl	not installed		
Seat <u>C</u> apillary: 400.0 ▼ μl			
Max. Inj. Volume: 500 µl	use BCD port for		
Multiple Draw:			
<u>W</u> aittime: 2 s	O Binary <u>D</u> utput		
Trays <u>A</u> : 15 Vials <u>B</u> : 15 Vials			
<u>O</u> K Cancel <u>H</u> elp			

Figure 2 Injector configuration window

Configuration Example 1: 100-µl sample loop and 400-µl seat capillary

- The 400-µl seat capillary was installed according to the "Installation Note for the Multi-draw Kit G1313-68711"
- The seat capillary was configured in the *Injector Configuration* window.
- Maximum injection volume: 500 µl (4 x 100 µl in the seat capillary and 100 µl in the sample loop).
- Draw speed: 200 µl/min, configured in the *Setup Injector* window of the ChemStation.
- Sample: Isocratic sample, diluted 1:100 with water/ acetonitrile 60:40.

Performance characteristics

Precision of peak area

After a certain number of injections of the same sample the relative standard deviation of the peak areas were determined. The relative standard deviation shows the reproducibility of the whole system, which is influenced by all system components. For precision measurements ten injections were performed from the same sample.

The relative standard deviation was calculated using the sequence summary report of the Chem-Station. Figure 3 shows the precision of the peak area.



Figure 3

Precision of peak area for configuration example 1

Linearity

Analysis of samples with different compound concentrations gives the linearity curve of the autosampler. The signals of the samples have to be in the linear range of the detector. For linearity measurements at least three injection volumes were injected. For each injection volume ten measurements were performed and the mean value was used for calculating the linearity correlation coefficient. Linearity is shown in figure 4.



Figure 4 Linearity for configuration example 1

Configuration Example 2: 100-µl sample loop and 1400-µl seat capillary

- The 1400-µl seat capillary was installed according to the *"Installation Note for the Multi-draw Kit G1313-68711"*.
- The seat capillary was configured in the *Injector Configuration* window.
- Maximum injection volume: 1500 µl (14 x 100 µl in the seat capillary and 100 µl in the sample loop).
- Draw speed: 200 µl/min (configured in the *Setup Injector* window of the ChemStation).
- Sample: Isocratic sample, diluted 0.7:100 with water/ acetonitrile 60:40.

Precision of peak area and linearity are shown in figures 5 and 6.

Chromatographic Parameters			
Mobile phase:	A = water		
	B = acetonitrile		
Gradient:	at 0 min 40 % B		
	at 10 min 80 % B		
	at 12 min 80 % B		
	at 13 min 40 % B		
Flow rate:	1 ml/min		
Stop time:	13 min		
Post time:	5 min		
Column:	Hypersil ODS		
	4 x 125 mm, 5 μm		
Temperature:	25 °C		
Detector:	DAD 254 nm/16		
	(reference:		
	360 nm/100)		









Precision of peak area for configuration example 2

Configuration Example 3: 900-µl sample loop and 2.3-µl seat capillary

- The 900-µl sample loop, the analytical head and the needle were installed according to the "Installation Note for 900-µl Injection Upgrade Kit G1363A".
- The sample loop was configured in the *Injector Configuration* window (syringe).
- Maximum injection volume: 900 µl.
- Draw speed: 200 µl/min (configured in the *Setup Injector* window of the ChemStation).
- Sample: isocratic sample, diluted 0.8:100 with water/ acetonitrile 60:40.

Precision of peak area and linearity are shown in figures 7 and 8.

Chromatographic Parameters Mobile phase: A = water B = acetonitrile Gradient: at 0 min 40 % B at 10 min 80 % B at 12 min 80 % B at 13 min 40 % B Flow rate: 1 ml/min Stop time: 13 min Post time: 5 min Column: Hypersil ODS 4 x 125 mm, 5 µm Temperature: 25 °C DAD 254 nm/16 Detector: (reference: 360 nm/100)









Precision of peak area for configuration example 3

Configuration Example 4: 900-µl sample loop and 1400-µl seat capillary

- The 900-µl sample loop, the analytical head and the needle were installed according to the "Installation Note for 900-µl Injection Upgrade Kit G1363A".
- The 1400-µl seat capillary was installed according to the "Installation Note for Multi-draw Upgrade Kit G1313-68711".
- The sample loop and the seat capillary were configured in the *Injector Configuration* window.
- Maximum injection volume: 1800 µl (using the injector program up to 2300 µl).
- Draw speed: 200 µl/min (configured in the *Setup Injector* window of the ChemStation).
- Sample: isocratic sample, diluted 0.4:100 with water/ acetonitrile 80:20.

Chromatographic Parameters

Mobile phase:	A = water B = acetonitrile
Gradient:	at 0 min 20 % B at 10 min 80 % B
	at 15 min 80 % B
	at 18 min 20 % B
Flow rate:	1 ml/min
Stop time:	18 min
Post time:	5 min
Column:	Hypersil ODS
	4 x 125 mm, 5 µm
Temperature:	25 °C
Detector:	DAD 254 nm/16
	(reference:
	360 nm/100)







Figure 10 Precision of peak area for configuration example 4





Configuration Example 5: 900-µl sample loop and 5000-µl seat capillary

- The 900-µl sample loop, the analytical head and the needle were installed according to the "Installation Note for 900-µl Injection Upgrade Kit G1363A".
- The 5000-µl seat capillary (GC sample loop 5cc, part number 0101-0301) was installed according to the "Installation Note for Multi-draw Upgrade Kit G1313-68711".
- The sample loop and the seat capillary were configured in the *Injector Configuration* window.
- Maximum injection volume: 5400 µl (5900 µl with injector program).
- Draw speed: 1000 µl/min (configured in the *Setup Injector* window of the ChemStation).
- Sample: Isocratic sample, diluted 0.4:100 with water/ acetonitrile 80:20.

Chromatographic Parameters

Mobile phase:	A = water
	B = acetonitrile
Gradient:	at 0 min 20 % B
	at 10 min 80 % B
	at 20 min 80 % B
	at 21 min 20 % B
Flow rate:	1 ml/min
Stop time:	21 min
Post time:	10 min
Column:	Hypersil ODS
	4 x 125 mm, 5 µm
Temperature:	25 °C
Detector:	DAD 254 nm/16
	(reference:
	360 nm/100)







Figure 13 Linearity for configuration example 5

Conclusion

The precision and linearity of peak area were measured for a Agilent 1100 Series autosampler configured with the multi-draw upgrade kit, the 900-µl injection upgrade kit, a combination of both, and a 5000-µl seat capillary. The results for the precision of area and linearity are summarized in table 2.

Precision of area and linearity show very good values even for high injection volumes of 1000– 4000 µl. The values become slightly inferior only for an injection volume of 5000 µl. Therefore, we demonstrated that the Agilent 1100 Series autosampler can be used for precise and reliable injection of high sample volumes.

Configuration	Precision of Area	Linearity
	(RSD)	(Correlation of coefficient)
Example 1	0.1 %	1.00000
Example 2	< 0.5 % (injection volume < 1000 µl) 0.5 % (injection volume > 1000 µl)	1.00000
Example 3	>0.5 % (injection volume < 5 µl) < 0.5 % (injection volume > 5 µl)	1.00000
Example 4	< 0.5 %, (biphenyl > 0.5 %, decomposing)	1.00000 (biphenyl <1.00000)
Example 5	< 0.5 % (injection volume < 2000 µl) 1 % (injection volume > 2000 µl) (biphenyl > 1 %, decomposing)	1.00000 (injection volume < 4000 µl) < 1.00000 (injection volume > 4000 µl)

Table 2 Overview of results

<u>Literature</u>

1

"Agilent 1100 Series: HPLC injection systems designed for precision and reliability" *Brochure Brief*, **1999**, publication number 5968-4467E

2

"Installation Note for Multidraw Upgrade Kit G1313-68711" *Installation Note*, **1997**, part number G1313-90100

3

"Installation Note for 900 µl Injection Upgrade Kit G1363A" *Installation Note*, **1997**, part number G1363-90100

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