

# High Volume Injection up to 5 mL with the Agilent 1200 Infinity Series Online SPE Solution for Highest Sensitivity

## Technical Overview

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### Abstract

The Agilent 1260 Infinity Standard Autosampler is recommended in the Agilent 1200 Infinity Series Online SPE Solution because it offers enhanced injection volume capabilities. High volumes are needed for highly sensitive measurements, like pesticides in water. With the 900  $\mu\text{L}$  injection upgrade kit and the multi-draw upgrade kit, up to 1,800  $\mu\text{L}$  can be injected.

This Technical Note describes an additional high volume injection kit with a 5-mL sample loop and presents data supporting the precision of peak area and linearity. The 5-mL sample loop is connected to an external 2-position/6-port valve, which makes it possible to switch the sample loop out of the flow path to run normal HPLC methods in direct inject mode without the delay volume of the external loop.



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## Introduction

To increase the injection volume of the 1260 Infinity Standard Autosampler, a 900- $\mu$ L analytical head and a 1,400- $\mu$ L seat capillary can be added. This expands the injection volume to a maximum of 1,800  $\mu$ L<sup>1</sup>.

However, for some online SPE methods and low compound concentrations, larger injection volumes are required to increase the sensitivity of the system and to achieve detection limits in the low ng/L range for applications such as drinking, environmental, and wastewaters<sup>2</sup>. Levels below 1 ng/L can be detected with the help of an external valve drive and a 5-mL sample loop. The sample is concentrated on the trapping column and the analytes are backflushed onto the analytical column. Using the solvent selection valve, the sample loop and trapping columns are flushed with up to three solvents with the 1290 Infinity Flexible Cube.

## Experimental and Instrumentation

All experiments were performed on an Agilent 1200 Infinity Online SPE Solution comprising:

- Agilent 1260 Infinity Binary Pump (p/n G1312B) and LAN card (p/n G1369C)
- Agilent 1260 Infinity Standard Autosampler (p/n G1329B) with 900- $\mu$ L head (p/n G1313-60007) and an Agilent 1290 Infinity Thermostat (p/n G1330B)
- Agilent 1290 Infinity Flexible Cube (p/n G4227A) with two 2-position/10-port valves
  - Agilent 1200 Infinity Online SPE starter kit: (p/n G4742A) (one 2-position/10-port valve, 600 bar)
  - Agilent 1200 Infinity Online SPE direct injection kit: (p/n G4744A) (one 2-position/10-port valve, 600 bar)
- External valve drive (p/n G1170A) with 2-position/6-port valve (p/n G4231A) and a 5-mL loop (p/n G2260-68711)
- Agilent 1290 Infinity Thermostated Column Compartment (p/n G1316C)
- Agilent Triple Quadrupole LC/MS System with Agilent Jet Stream Technology (p/n G6460A)

## Trapping columns

- Two Guard Column Hardware Kits (p/n 820999-901) (as part of G4742A)
- PLRP-S Cartridges, 2.1  $\times$  12.5 mm, 15–20  $\mu$ m (p/n 5982-1271) (as part of G4742A)
- 6-mL screw cap vials (glass p/n 9301-1377, screw caps p/n 9301-1379, pre-slit septa p/n 5188-2758)

## Software

- Agilent MassHunter Data Acquisition for Triple Quadrupole Mass Spectrometer, Version 06.00
- Agilent MassHunter Optimizer software, Version 06.00
- Agilent MassHunter Source and iFunnel Optimizer software, Version 06.00
- Agilent MassHunter qualitative software, Version 06.00
- Agilent MassHunter quantitative software, Version 05.02

## System configuration and principle of operation

The configuration of the system is based on the Agilent 1200 Infinity Series Online SPE Solution. This includes the 1290 Infinity Flexible Cube, which hosts two 2-position/10-port valves for direct injection and online SPE methods<sup>3</sup> on the same instrument. To upgrade the instrument for high volume injection with up to 5 mL, an additional external valve drive can be installed (G1170A) at the 1290 Infinity Flexible Cube (Figures 1 and 2). The external valve hosts a 2-pos/6-port valve to switch the sample loop in or out of the flow path. The sample loop is placed in the flow path between the needle seat capillary and the injection valve (port 5) of the autosampler.

Figure 1 shows the valve setup with two 2-position/10-port valves and one 2-position/6-port valve, which hosts the 5-mL loop on the external drive.

After the loading process, the external loop is flushed with the 1290 Flexible Cube. If lower injection volumes are sufficient, the external loop can be easily bypassed due to the 2-position/6-port valve on the external drive (Figure 2).

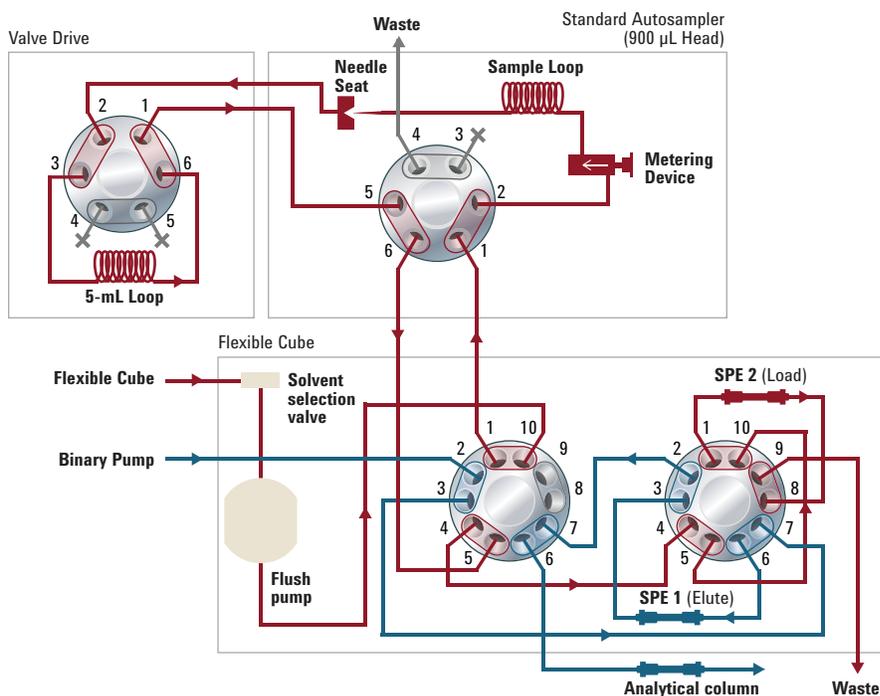


Figure 1. The Agilent 1290 Infinity Flexible Cube with two 2-position/10-port valves, the autosampler configuration, and the external valve drive with the 5-mL loop. The sample is drawn and ejected with the autosampler into the external loop. After the ejection, the 1290 Infinity Flexible Cube loads the samples from the loop to one of the trapping columns (see flow path).

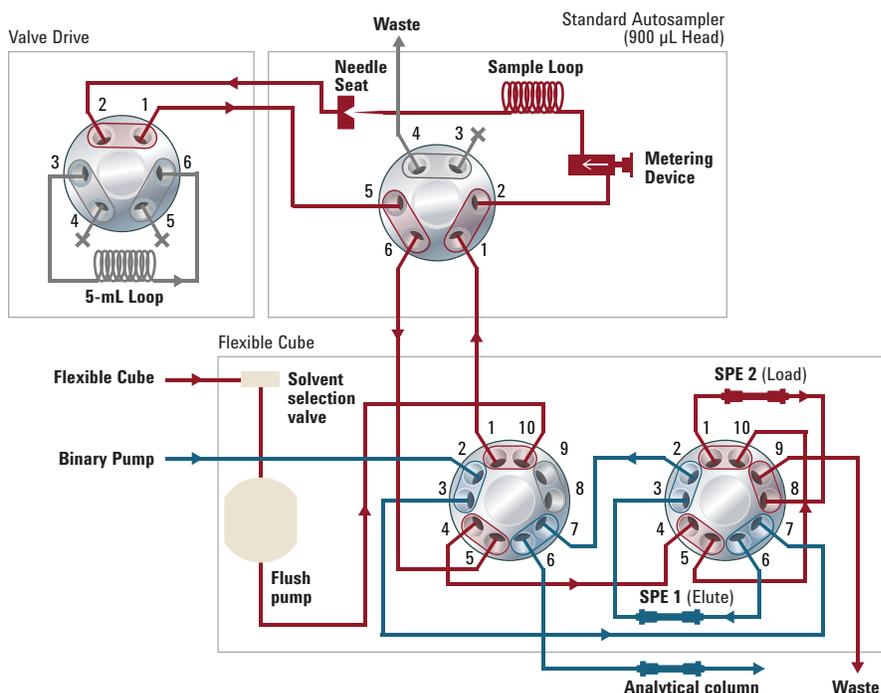


Figure 2. The 5-mL loop can be easily disabled if the position of the 2-position/6-port valve is changed. Hence, additional dead volume is saved.

Finally, the system is coupled to an Agilent 6460 triple quadrupole mass spectrometer.

The online SPE procedure consists of three steps (Figure 3):

1. Loading and enriching the sample on the SPE cartridge (SPE 1)
2. Elution of SPE 1 with the gradient flow
3. Rinsing and reconditioning SPE 2 (from the previous run)

### Samples

Tap water was spiked with an equimolar mixture of five pesticides (carbofuran, dimethoate, imazalil, metazachlor, metosulam) with 50 ng/L. Six consecutive runs were performed for every injection volume.

### Chemicals

All solvents used were LC/MS grade. Acetonitrile was purchased from Merck, Germany. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with LC-Pak Polisher and a 0.22- $\mu$ m membrane point-of-use cartridge (Millipak). Formic acid was purchased from Agilent Technologies (p/n G2453-85060).

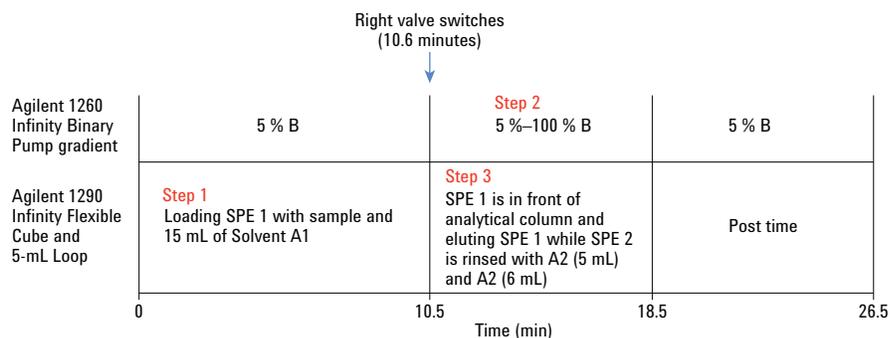


Figure 3. Schematic of the online SPE method, which includes the Agilent 1290 Infinity Flexible Cube as a loading pump and an Agilent 1260 Infinity Binary Pump to run the gradient.

### Method

1260 Infinity Binary Pump	
Solvent A	water, 0.1% formic acid
Solvent B	95% acetonitrile, 0.1% formic acid
Flow rate	0.5 mL/min
Gradient	Time (min)    5 % B
	0                    5
	10.5                5
	16.5                100
	18.5                100
Post time	8 minutes
1290 Infinity Thermostatted Column Compartment	
Temperature	30°C
Column	
Agilent ZORBAX Eclipse Plus C18, 2.1 × 150 mm, 3.5 $\mu$ m (p/n 959763-902)	
1290 Infinity Flexible Cube	
Pump	1.5 mL/min
Solvent selection valve	Solvent A1: Water, Solvent A2: Acetonitrile
1260 Infinity Autosampler	
Draw and eject speed	900 $\mu$ L/min

## Results and Discussion

### Performance characteristics – precision of peak area

Seven different injection volumes (5, 4.5, 4, 3, 2, 1, and 0.5 mL,  $n = 6$ ) were analyzed and the relative standard deviation (RSD) of the peak areas were calculated. Figure 4 shows the RSD values for different injection volumes and compounds.

The chemical properties of the analytes have a huge impact on the linearity and precision. As it can be seen in Figure 4, the most nonpolar and latest eluting compound, matazachlor, has area RSD values of less than 2 % for all injection volumes. In comparison, the first eluting compound, dimethoate, has the highest area RSD values in this set of compounds (0.7–3.6 %). For all compounds and injection volumes the area RSD was < 4 %.

### Performance characteristics – linearity

Analysis of samples with different injection volumes but the same compound concentrations gives the linearity of the system coupled to the external sample loop. The calibration curves were developed for seven levels, at the same sample concentrations, and the linearity is illustrated by the correlation coefficients listed in Table 1. The linearity of the analyte is dependent upon its chemical characteristics, its ability to bind to the trapping column, and the performance of the autosampler coupled to the external loop.

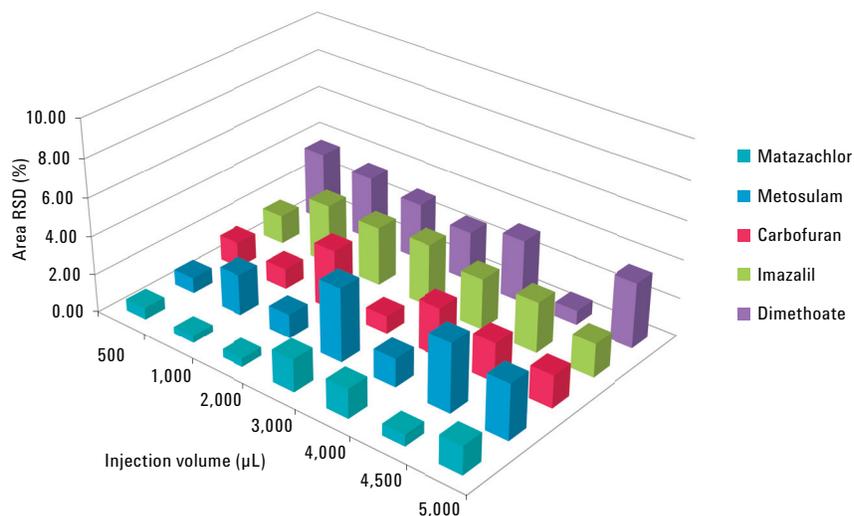


Figure 4. Area RSD and its correlation to the injection volume (area RSDs for all compounds < 4 %).

Table 1. Compounds and their correlation coefficient and retention times.

Compound	Coefficient of determination ( $R^2$ )	Retention time (min)
dimethoate	0.992	13.8
imazalil	0.990	14.5
carbofuran	0.994	14.8
metosulam	0.955	15.1
matazachlor	0.995	15.4

Figure 5 shows the correlation of peak area and injection volume for two pesticides. Regarding the peak area, it can be observed that matazachlor has, compared to the other analytes, the best characteristic to bind on the trapping columns. In addition, the peak area and sensitivity increases steadily to an injection volume of 4,500  $\mu\text{L}$ . Between 4,500 and 5,000  $\mu\text{L}$ , the peak area remains constant. The same behavior can be seen for all other compounds. This is a well-known behavior of fixed-loop injectors. When filling the loop to more than 50–75 % of the loop volume, some of the sample is already flushed out the other end of the loop due to the parabolic flow profile. Therefore, it is recommended not to fill the loop more than 4.5 mL.

**Tip**

The injector program can be optimized to save time during the autosampler multidraw process. The autosampler needs time to draw the sample from the vial and eject it into the loop. Therefore, it is more efficient to draw 900  $\mu\text{L}$  in the last step of the injector program, because this volume does not have to be injected into the loop again (Figure 6). Another advantage of this injector program is that the 900  $\mu\text{L}$ , which is drawn in the last step, will not be ejected in the loop. This minimizes the effect of the parabolic flow profile, since the loop is filled with less volume.

During method optimization, it was determined that at least one loop volume of solvent A1 (water) in the 1290 Infinity Flexible Cube should be used to transfer the sample from the sample loop to the SPE cartridge. However, the best performance and reproducibility was achieved when the sample loop was flushed three times (15 mL) to transfer the sample to the cartridge.

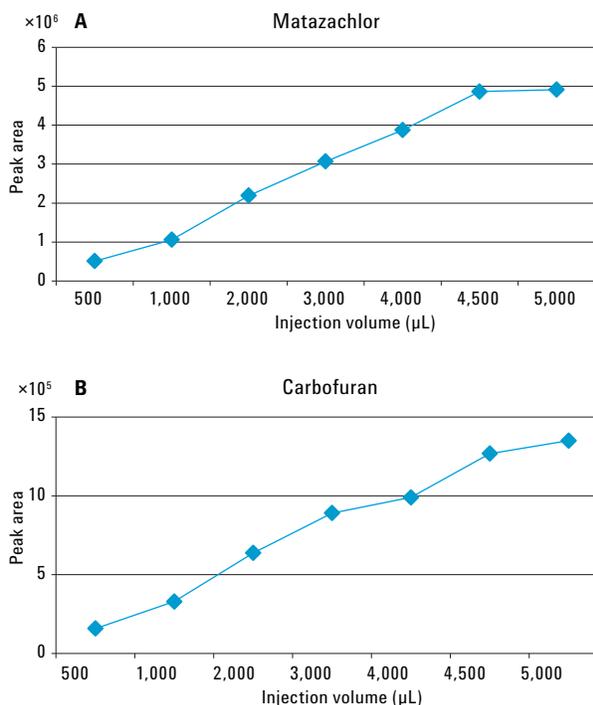


Figure 5. Correlation of the peak area and the injection volume of matazachlor and carbofuran (n = 6).

Properties		DA	Sampler	Sampler Pretreatment	Binary Pump	Column Comp.	Valve	Flexible Cube	QQQ
<input checked="" type="checkbox"/> Use Injector Program									
Function	Parameter								
Draw	Draw 400 $\mu\text{L}$ from sample with default speed using default offset								
Eject	Eject maximum volume to seat with default speed using default offset								
Draw	Draw 900 $\mu\text{L}$ from sample with default speed using default offset								
Eject	Eject maximum volume to seat with default speed using default offset								
Draw	Draw 900 $\mu\text{L}$ from sample with default speed using default offset								
Eject	Eject maximum volume to seat with default speed using default offset								
Draw	Draw 900 $\mu\text{L}$ from sample with default speed using default offset								
Eject	Eject maximum volume to seat with default speed using default offset								
Draw	Draw 900 $\mu\text{L}$ from sample with default speed using default offset								
Inject	Inject								

Figure 6. Screenshot of injector program for 4-mL injection volume.

## Conclusion

This Technical Overview shows the precision and linearity of the Agilent 1200 Infinity Series Online SPE Solution with a high volume injection kit, consisting of an external valve drive with a 5-mL sample loop. It was demonstrated that the 5-mL sample loop can be easily integrated in the 1200 Infinity Series Online SPE Solution and achieves precise and reliable injection volumes between 500 and 4,500  $\mu\text{L}$ .

## References

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2. R. López-Serna, S. Pérez, A. Ginebreda, M. Petrovic, D. Barceló, "Fully automated determination of 74 pharmaceuticals in environmental and waste waters by online solid phase extraction–liquid chromatography–electrospray–tandem mass spectrometry", *Talanta* 83, 410–424, **2010**.
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