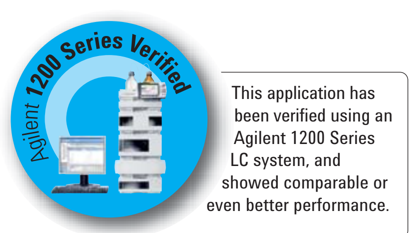
The Agilent 1100 Series dual-loop autosampler PS – Optimum performance when injecting large sample volumes

Technical Note



Abstract

This Technical Note describes the configuration and operating principles of the Agilent 1100 Series dual-loop autosampler, as well as the different injection modes – complete and partial loop fill. Para-meters influencing the quality of the injection are shown for both injection methods. Carry over is discussed and different wash and rinse features are explained to keep the carry over as low as possible.

Agilent Technologies

Introduction

The Agilent 1100 Series dual-loop autosampler PS (DLA) is an essential part of the Agilent 1100 Series purification platform[1] but is also an ideal tool for the injection of samples from sample vials or wellplates in analytical HPLC when higher injection volumes are required. The injection principle of the DLA is different to that of other Agilent 1100 Series autosamplers. In all other autosamplers the sample is drawn into the sample loop and then the sample loop, together with the needle and the metering device, is switched into the high-pressure flow path from the pump to the column. In the DLA however, the sample is drawn into a buffer loop and then transferred into a sample loop using a low-pressure metering device. Then the sample loop is switched into the high-pressure flow path[2]. This so-called fixed loop injection principle allows two modes of operation:

- partial loop fill, where a sample volume smaller than the sample loop volume is injected without loosing any sample, and
- complete loop fill, with high precision which sacrifices most of the drawn sample volume.

Partial loop fill is the method of choice for preparative work while complete loop fill is used for analytical tasks[3].

Equipment

The experiments were performed on an Agilent 1100 Series system containing the following modules:

- Two Agilent 1100 Series preparative pumps
- Agilent 1100 Series dual-loop autosampler PS
- Agilent 1100 Series column organizer
- Agilent 1100 Series diode array detector

The system was controlled using the Agilent ChemStation (rev. B.01.01).

Operating principle

Hardware configuration

Figure 1 shows the important parts of the DLA:

1. Metering device (5 mL) for the injection of high sample volumes in a single stroke
2. 3-way valve and flush solvent for washing the needle seats and the injection valve
3. Two needle seats, one for each sample loop
4. 2-position/10-port valve with two sample loops
5. Peristaltic pump for the needle wash

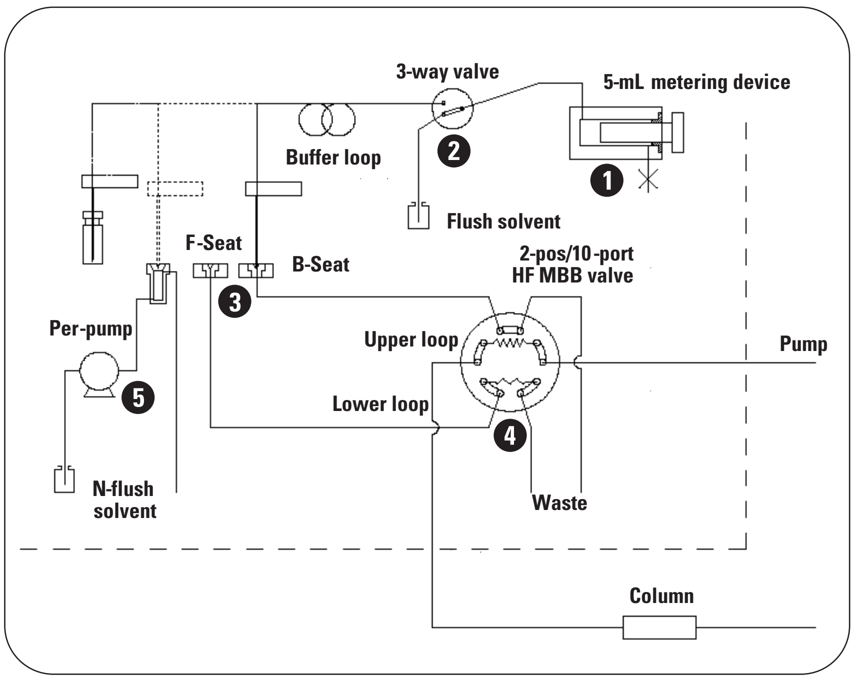


Figure 1
Schematic drawing of the DLA

As mentioned before, the operating principle of the DLA is different from all other Agilent 1100 Series autosamplers. One of the sample loops is filled using the low-pressure metering device and afterwards the sample loop is switched into the high-pressure flow path. Figure 2 shows the basic steps of operation, which include:

A. The sample is drawn into the needle and the buffer loop (figure 1) using the metering device.
B. The sample (black line) is ejected into one of the sample loops using the metering device.
C. The sample loop is switched into the high-pressure flow path from the pump to the column.
D. During the run the needle seat and the 2-position/10-port valve are washed using the flush solvent (dotted line).

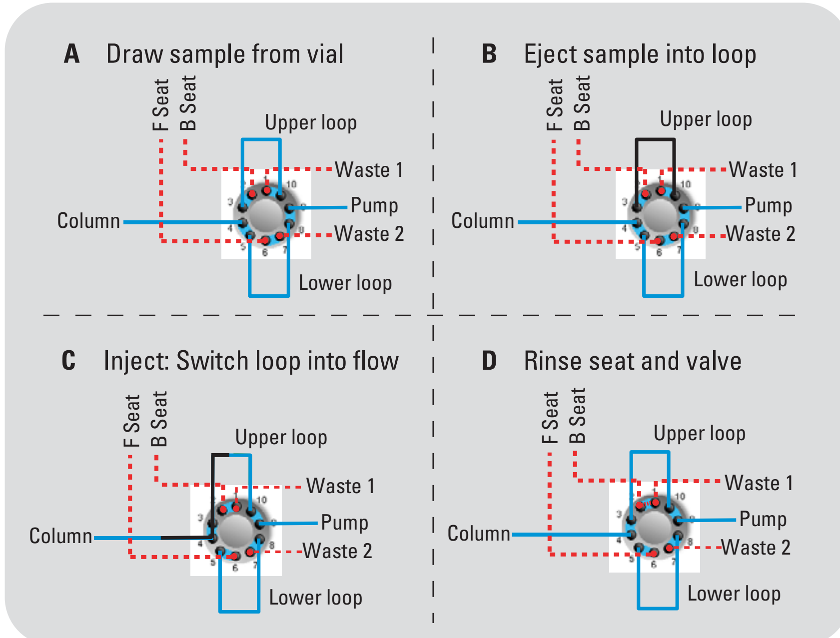


Figure 2
Injection principle

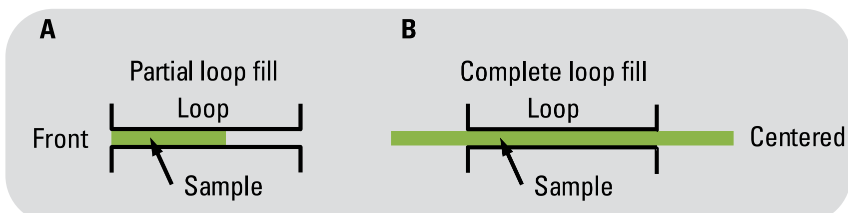


Figure 3
Partial and complete loop fill

Complete and partial loop fill

The fixed-loop concept of the DLA allows two modes of operation – complete and partial loop fill. When high injection precision is required complete loop fill must be used, which means that a sample volume higher than the sample loop volume must be drawn from the sample container. Most of the sample volume is used to flush the sample loop, which ensures that the loop is completely filled with the sample. In other words, most of the drawn sample volume is lost.

If injection precision is not crucial and it is important that no sample is wasted, partial loop fill is the method of choice. A sample volume smaller than the sample loop volume is drawn from the sample container and ejected into the loop.

In partial loop fill the drawn sample volume is pushed to the front of the sample loop while in complete loop fill the sample is centered in the sample loop (figure 3).

Summary of the advantages and disadvantages of complete and partial loop fill:

- Complete loop fill
  - Loop must be overfilled (by factor 3 to 5), most of the sample goes to waste
  - High precision

→ Ideal for analytical tasks

- Partial loop fill
  - Complete sample is injected onto the column
  - Lower precision (depends on sample loop size, sample volume, draw speed, etc.)

→ Ideal for preparative tasks

Performance

1. Complete loop fill

Complete loop fill is the method of choice for highest injection precision which is required for analytical HPLC. The injection volume is determined by the size of the sample loop, however for best performance an overfill factor must be specified in the software. Using the minimum overfill factor of 1 will not lead to accurate and precise results[4]. In figure 4 the peak area is shown for different overfill factors from 1 to 7 (50-µL sample loop). It can be seen that the peak area strongly increases up to an overfill factor of 3 to 5. This means that with overfill factors below 3 the sample loop is not completely filled. The reason why overfilling is necessary is the hydrodynamic behavior of fluids as they pass through tubing. A process called laminar flow takes place under conditions in which molecules close to the tubing walls are slowed by frictional forces. The result is a bullet-shaped profile in which the molecules in the center of the stream travel roughly twice the velocity of those at the tubing wall[4]. This also leads to a lower area precision for repetitive injections.

Note: To achieve highest accuracy and precision an overfill factor of at least 3 to 5 must be used for complete loop filling.

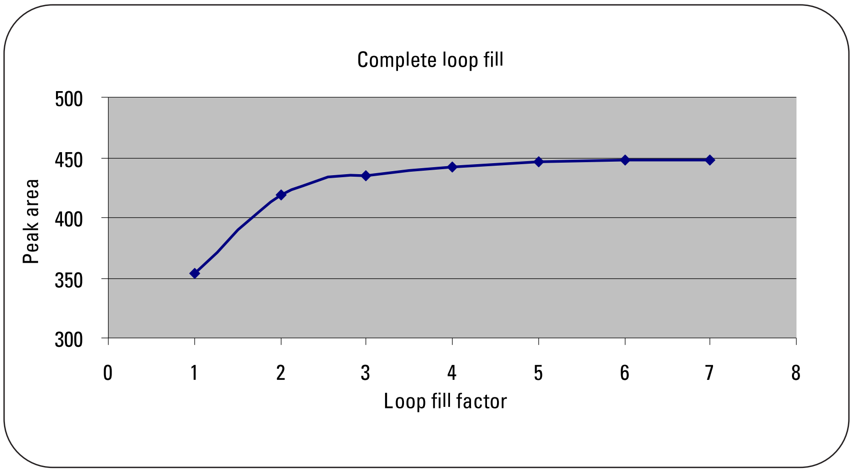


Figure 4
Peak area for different overfill factors

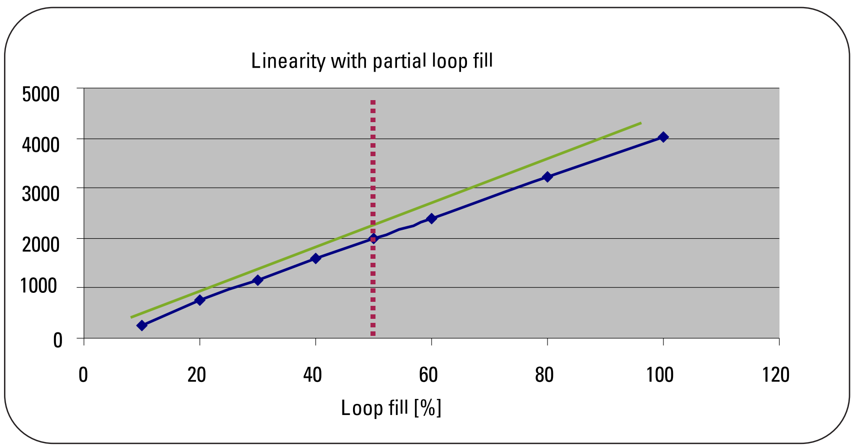


Figure 5
Linearity of peak area for partial loop fill

2. Partial loop fill

The objective of the injection in preparative HPLC is to apply the entire sample drawn from the sample container to the column while injection precision is only of minor interest. However, in the next experiments the injection precision is used as a parameter for the quality and reliability of the injection. In complete loop fill the overfill factor is the most important parameter influencing the injection precision; in partial loop fill several parameters have an influence on the quality of the injection. These parameters are:

- sample loop fill factor
- sample volume
- draw and eject speed

Sample loop fill factor

Figure 5 shows the result of several injections with different injection volumes using the same sample loop (500 µL). The peak area increases linearly until the loop is filled approximately up to 50 %, marked by the dotted line. This means that in order to minimize the sample loss the maximum injection volume should not be

more than 50 % of the sample loop volume.
Note: To make sure the entire-sample drawn from the sample container is applied to the column the sample loop should not be filled more than 50 % of the sample loop volume.

Sample volume

The sample volume should not be too low compared to the volume of the sample loop (1000 µL) to achieve good injection precision in partial loop fill (figure 6). A good area precision of < 0.5 % was achieved by filling the 1000-µL loop with at least 50–100 µL.
Note: To attain the highest area precision and good injection quality the sample loop should be filled with 10–50 % of the sample loop volume when using partial loop fill.

Draw and eject speed

The draw and eject speed for the sample also influence the quality of the injection when using partial loop fill. In the example shown in figure 7, 500 µL of sample were injected using a 1000-µL sample loop. The area precision is well below 1 % for all draw and eject speeds. Despite the draw and inject speed having no influence on area precision, there is an influence on the recovery as shown in figure 8.
Note: To achieve the best area precision the draw and eject speed should not be set too high.

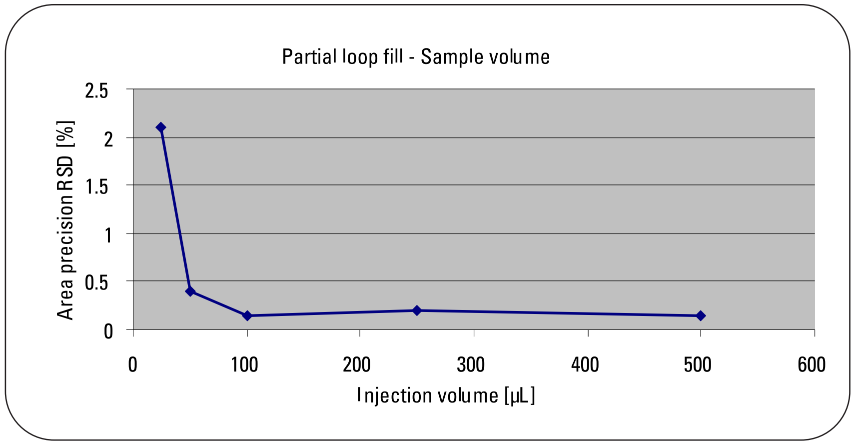


Figure 6
Area precision for different sample volumes

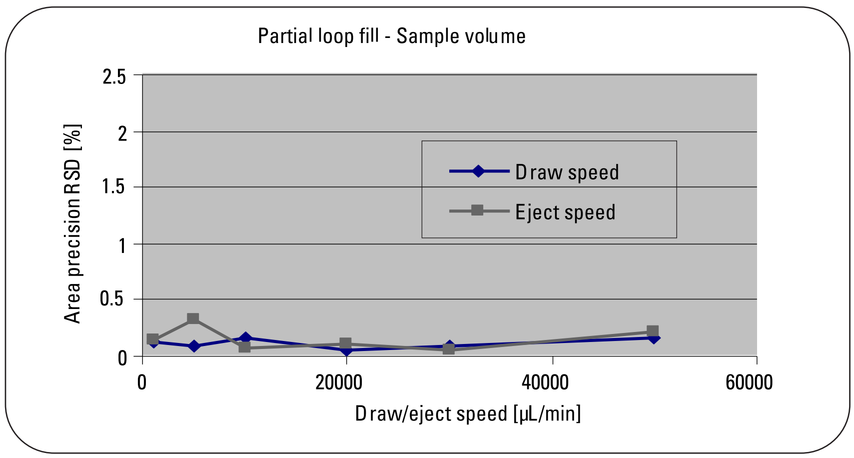


Figure 7
Area precision for different draw and eject speeds

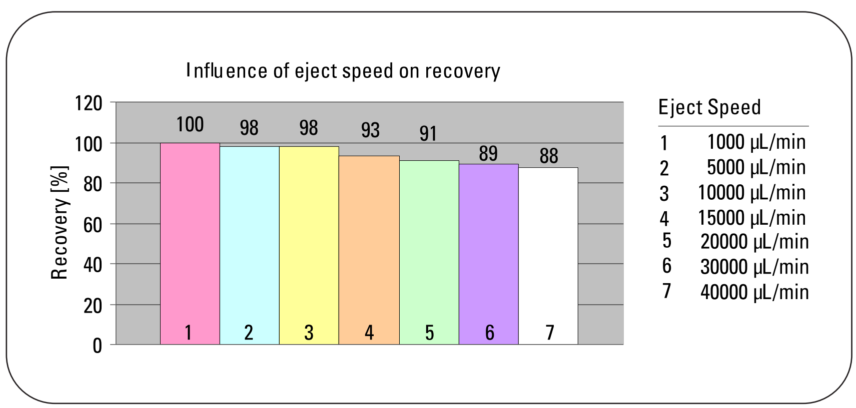


Figure 8
Partial loop fill performance – influence of draw/eject speed

Carry over

The dual-loop autosampler has several features to minimize carry over. After the sample is drawn into the needle the outside of the needle can be cleaned either by dipping it into a wash vial or by actively washing the needle with an appropriate solvent using the peristaltic pump (figure 1). The carry over can be lowered even further by washing the needle interior, the needle seat and the injection valve after the injection using the wash solvent delivered by the metering device. Figure 9 shows the influence of the different flushing features. The sample used for the experiments contained four compounds of low polarity with a concentration of 2–12mg of each compound in 1000-µL sample solvent (partial loop fill, 2000-µL loop).

Note: In general, the carry over depends on several factors, for example, compound, sample concentration, flush and rinse mechanisms, and flush solvent. However, it can be said that washing the needle exterior with an appropriate solvent after the sample was drawn and rinsing the needle seat and the injection valve after the injection leads to the lowest carry over.

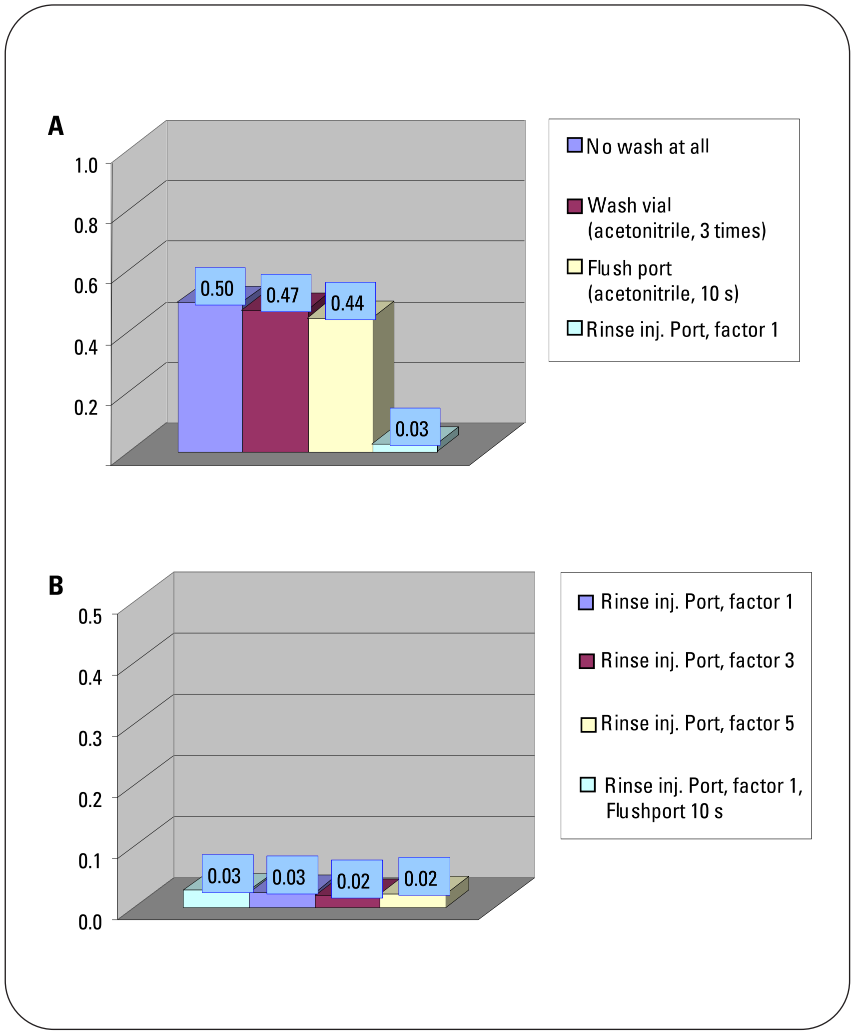


Figure 9
Carry over results for different flushing features

Conclusion

The following should be kept in mind to achieve the best performance of the Agilent 1100 Series dual-loop autosampler:

Complete loop fill

- For the highest area precision and best analytical results the complete loop fill method must be used.
- To achieve the best injection accuracy and precision the overfill factor should be between 3 to 5.

Partial loop fill

- Partial loop fill must be used for preparative work, where the entire sample drawn from the sample container must be applied to the column.
- For best injection accuracy and highest sample recovery the sample loop should not be filled with more than 50 % of the sample loop volume.
- For best injection precision using partial loop fill, the ratio of sample volume to sample loop volume should be between 0.25:1 and 0.5:1.
- Reducing the draw and eject speed leads to higher recovery.

References

1.
"New perspectives in purification with HPLC and HPLC/MS", *Agilent Technologies brochure, publication number 5988-3673EN*, **2001**.

2.
"Maintaining Autosampler Performance" LC/GC International", *John W. Dolan, LC/GC International, 7, 418-422*, **1997**.

3.
"Autosamplers, Part 1 – Design Features", *John W. Dolan, LC/GC North America, 4, 386-391*, **2001**.

4.
"Injection Loop Adsorption", *John W. Dolan, LC/GC International, 4, 530-533*, **1996**.

www.agilent.com/chem/1200

Published April 1, 2007
Publication Number 5989-1714EN

Agilent Technologies