



Tips and Tricks for Achieving Near-Zero Carryover Using the Multiwash Function of the Agilent 1290 Infinity II Multisampler

Technical Overview

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Abstract

The Agilent 1290 Infinity II Multisampler sets new benchmarks in flexibility and productivity, and includes many user-selectable options and features such as the multiwash function. Using the multiwash capability, sample carryover originating from the autosampler can be reduced to less than 10 parts per million, or even eliminated completely. The outside surfaces of the injection needle and the needle seat can be flushed with up to three solvents, reducing carryover to a minimum. This Technical Overview describes tips and tricks on how to optimally use the multiwash function of the 1290 Infinity II Multisampler to reduce carryover.



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Principle of Operation

The reduction or elimination of sample carryover and cross-contamination has become a major challenge in LC and LC/MS analysis. The Agilent 1290 Infinity II Multisampler is optimized for lowest carryover by careful design of the flow path and use of materials that minimize sample adsorption.

Method settings of the Multisampler allow the user to set appropriate parameters to minimize carryover in any application. Autosampler-related carryover can occur at the inside or outside surfaces of the needle, the needle seat, and the injection valve. The following functions of the Multisampler can be used to minimize carryover:

- Internal needle wash
- External needle wash
- Needle seat backflush
- Injection valve cleaning

As the Multisampler has a flow-through injection needle design, the needle is part of the fluid path during operation. Additionally, the outside of the needle can be cleaned and rinsed in a flush port with up to three solvents for a user-defined duration. A peristaltic pump (piezo pump) delivers fresh wash solvents to the flush port. Seat backflush is enabled by a second pump, an integrated high-pressure flush pump.

General Recommendations

For efficient reduction of carryover, it is important that the sample be soluble in weak and strong wash solvents. Typically, the solvent in which the sample is dissolved is an efficient solvent for flushing. In addition, the flushing procedure can contain weak as well as strong solvents.

Priming and Cleaning the Agilent 1290 Infinity II Multisampler

For priming and cleaning the 1290 Infinity II Multisampler outside of an analytical run, the user can select the Auto-clean function with a right click in the user interface. Here, the user can prime all solvents and wash the needle, seat, injection valve, and flow path for a user-defined duration (Figure 2).

IMPORTANT: It takes approximately 30 seconds to fully exchange one solvent for another in the flush port. To flush and exchange the solvent in the seat, it takes 18 seconds. Additionally, it is strongly recommended to use the Auto-clean function to flush the 1290 Infinity II Multisampler regularly with all installed solvents.

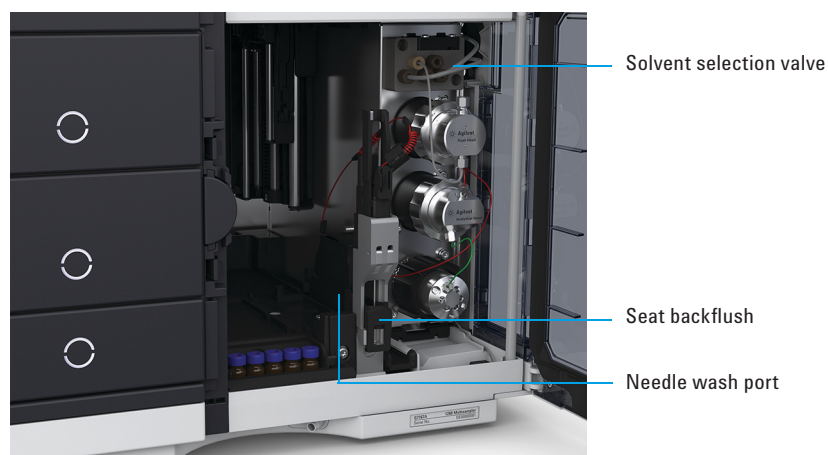


Figure 1. Agilent 1290 Infinity II Multisampler equipped with multiwash option, which includes a solvent selection valve for the use of up to three wash solvents, a needle wash port, and seat backflush.

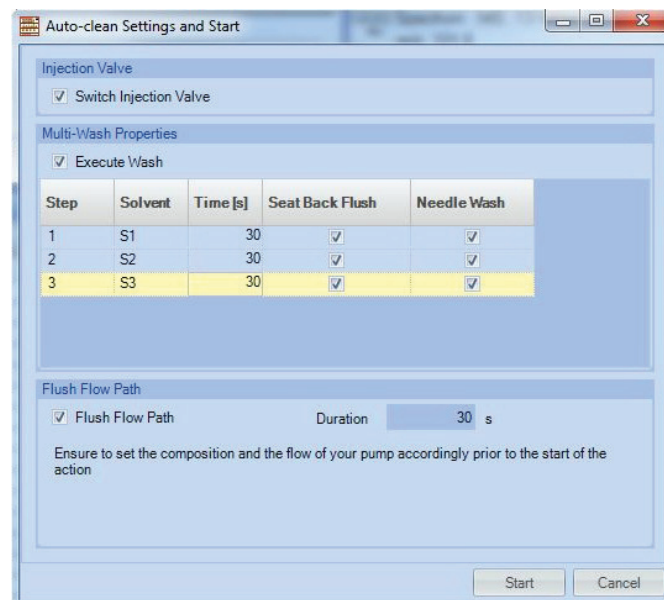


Figure 2. Priming and Auto-clean settings of needle, needle seat, flow path, and injection valve of the Agilent 1290 Infinity II Multisampler.

Setting Up a Multiwash Method

The user can define up to four steps and three solvents for the cleaning method, as well as the order and duration of the wash solvents. Figure 3 shows an example of three solvents being used (acetonitrile, 2-propanol, and water as part of the initial conditions). The user can select seat backflush, needle wash, or both, and can define the wash duration for every solvent separately, except the initial conditions. Simultaneous needle wash and seat backflush can only be done with one solvent. It is not possible to wash and flush the system with two different solvents at the same time.

TIP: When using short wash times for each solvent (less than 30 seconds), different layers of wash solvent will be formed in the delivery tubing. Even short wash times of 5 seconds for each wash solvent are sufficient for cleaning, if afterwards the box with **Start Conditions** is selected (Figure 3). With this feature, all solvents in the tubing will be transported to the needle and seat. Afterwards, all strong wash solvents will be completely exchanged, and will ensure that the instrument is ready for the analytical run. This is especially important for seat backflush. If seat backflush is used, the weak wash solvent should flush the strong wash solvent out of the seat capillary before injection to avoid poor peak shape and retention during the analytical run (Figure 4).

RECOMMENDATION: For best performance and chromatographic results, the weak wash solvent should be similar or identical to the initial gradient, and should be the last cleaning step to avoid poor peak shape (Figure 4). In addition, the strong wash solvent should be at least equal to the final elution composition of the gradient.

It is important that the organic and aqueous wash solvents are compatible with the mobile phase, sample, and instrumentation. For reversed phase chromatography, the wash solvents are usually pure acetonitrile or a mixture with methanol and water, or even isopropanol. It often helps to add some acid or base. For more information about the biocompatibility of the piezo pump, which contains polyether ether ketone (PEEK) material, with different solvents

and chemicals, please refer the User Manual¹. To create an efficient method, it is recommended to start with flushing durations of approximately 5 seconds for each solvent. In the following, check the **Start Condition** checkboxes as already described (Figures 3 and 4). If the result is not acceptable, increase the durations successively.

Finally, carryover is not only caused by the autosampler, but also by improper elution of the compound from the column. Therefore, a sufficient gradient time has to be selected. To eliminate the column as a source of carryover, it can be replaced by a restriction capillary to check whether carryover still occurs. Another source of carryover can be improper capillary connections that create dead volumes and trap analytes.

Multi-wash					
Step	Solvent	Time [s]	Seat Back Flush	Needle Wash	Comment
1	S1	3	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	H2O
2	S2	8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ACN
3	S3	10	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	2-Propanol
Start Cond.	S1		<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	

Figure 3. Example of a cleaning method with four steps and three wash solvents.

Multi-wash					
Step	Solvent	Time [s]	Seat Back Flush	Needle Wash	Comment
1	Off		<input type="checkbox"/>	<input type="checkbox"/>	
2	S2	8	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	ACN
3	S3	5	<input type="checkbox"/>	<input checked="" type="checkbox"/>	2-Propanol
Start Cond.	S1		<input checked="" type="checkbox"/>	<input type="checkbox"/>	

Figure 4. Example of a cleaning method with three steps and three wash solvents. Here, 2-propanol is used as a last step for washing the needle, and will remain in the needle wash port. In the analytical flow path, acetonitrile will be exchanged with water (initial start condition for seat backflush).

References

1. Agilent 1290 Infinity Multisampler User Manual, *Agilent Technologies*, publication number G7167-90000, **2014**.

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