Minimizing Sample Carryover Using the Multiwash Function of the Agilent 1290 Infinity II Multisampler

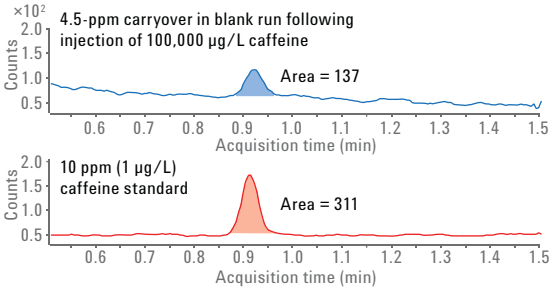
Technical Overview

Author

Bettina Schuhn
Agilent Technologies, Inc.
Waldbronn, Germany

Abstract

The Agilent 1290 Infinity II multisampler with multiwash achieves ultralow carryover for most compounds. This integrated feature flushes the outside surfaces of the injection needle with up to three different solvents, and backflushes the needle seat at the same time to prevent carryover. These two washing procedures reduce carryover for most compounds to less than 9 parts per million (ppm), which is shown here for caffeine and various pesticides using detection with a highly sensitive Agilent 6490 triple quadrupole mass spectrometer.



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Introduction

Sample carryover is an issue for LC, especially LC/MS analysis, and can lead to reduced sample throughput and impact data quality. The causes of carryover are often in the autosampler, where the injection takes place[1]. To avoid, or at least reduce carryover from one sample to the next, it is important to clean the inside and outside surfaces of the injection needle as well as the needle seat. The Agilent 1290 Infinity II multisampler with the optional multiwash feature offers this type of cleaning.

The flow-through principle of the 1290 Infinity II multisampler means the inside of the needle is flushed constantly. The outside surfaces of the needle are cleaned with the choice of three different solvents, which can be programmed with different washing times. In addition, the needle seat can be backflushed with these three solvents[2].

To determine sample carryover of caffeine and different pesticides, a 1290 Infinity II multisampler configured with multiwash was coupled to a highly sensitive Agilent 6490 triple quadrupole mass spectrometer. Different experiments demonstrated that the source of carryover is not only the autosampler, but also the analytical column or an installed switching valve. However, with an optimized washing procedure of the injection needle and seat, the carryover caused by the autosampler can be reduced to less than 9 ppm for many analytes.

Design of the Agilent 1290 Infinity II Multisampler

The 1290 Infinity II multisampler provides high flexibility in sample logistics through high sample capacity, even with different vial sizes. Lowest carryover is achieved using the multiwash feature, which allows the user to choose three different washing solvents for seat backflush and needle wash. More information is presented in a separate Agilent publication[2].

Experimental

Instrumentation

- Agilent 1290 Infinity II high-speed pump (2x G7120A)
- Agilent 1290 Infinity II multisampler with 40-µL metering device (G7167B)
- Agilent 1290 Infinity II multicolumn thermostat (G7116B)
- Agilent Quick-Change 2-position/10-port valve, 1,200 bar, with valve head (5067-4118)
- Agilent 1290 Infinity II diode array detector with 10-mm cell (G7117B)
- Agilent 6490 triple quadrupole LC/MS system with Agilent Jet Stream technology (G6490A)

Software

- Agilent MassHunter data acquisition software for triple quadrupole mass spectrometer, version 07.00, SP1
- 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 07.00

Samples

A pesticide mix (p/n 5190-0469) and a caffeine standard kit for LC/MS OQ/PV (p/n 8500-6917) were purchased from Agilent Technologies.

Chemicals

All solvents used were LC/MS grade. Methanol and acetonitrile were purchased from Sigma-Aldrich, USA. Fresh ultrapure water was obtained from a Milli-Q Integral system equipped with a LC-Pak Polisher and a 0.22-µm membrane point-of-use cartridge (Millipak). Formic acid (p/n G2453-85060) and ammonium formate (G1946-85021) were purchased from AgilentTechnologies.

Methods

Table 1. Caffeine method.

Parameter	Description
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (959757-902)
Mobile phase	A) Water + 0.1 % formic acid B) Acetonitrile + 0.1 % formic acid
Isocratic	11 %B for 1.5 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Washing procedure	10 seconds with water, 0.1 % formic acid (needle wash and seat backflush)
Column temperature	50 °C

Table 2. Pesticide method.

Parameter	Description
Column	Agilent ZORBAX Eclipse Plus C18, 2.1 × 50 mm, 1.8 µm (959757-902)
Mobile phase	A) Water + 0.1 % formic acid, 5 mM ammonium formate B) Methanol
Gradient	0 minutes, 5 %B 0.5 minutes, 5 %B 8 minutes, 95 %B 11 minutes, 95 %B
Post time	3 minutes
Flow rate	0.5 mL/min
Injection volume	1 µL
Washing procedure	See Table 3
Column temperature	Room temperature

Results and Discussion

Reduction of caffeine carryover

To demonstrate the ultralow carryover, the system was stressed with a very high concentration of 1 µL of 100,000 µg/L caffeine. To treat the multiplier in the MS with care, the high concentration of standard was not introduced into the MS, but guided to waste. The cleaning procedure of the 1290 Infinity II multisampler comprised flushing the needle and seat for 10 seconds with mobile phase A. The calculation for the concentration of carryover of caffeine was as follows:

Absolute concentration of caffeine standard	Relative concentration of carryover
100,000 µg/L	1,000,000 ppm
100 µg/L	1,000 ppm
1 µg/L	10 ppm
0.1 µg/L	1 ppm

The highest possible relative concentration was set to 1,000,000 ppm, and represents an absolute concentration of 100,000 µg/L. For the carryover calculation, a calibration curve was created in the range of 0.25 to 50 µg/L. For carryover determination, the following sequence was used:

1. Aqueous blank
2. Calibration (0.25 to 50 µg/L)
3. Aqueous blank before carryover test (n = 3)
4. 100,000 µg/L Caffeine injection
5. Aqueous carryover blank (n = 5)
6. Quality Control Standard

The first and third blanks in the sequence will show if the system is clean and free of analytes. With the calibration in the lower concentration range, the carryover of the ultrahigh concentrated standard can be quantified. After the 1-µL injection of the 100,000-µg/L standard, five blank injections of water were done to measure the amount of carryover, and determine when the system was clean again. Finally, a quality control standard was measured within the sequence.

The caffeine carryover was determined to be 4.5 ppm after the 100,000-µg/L standard injection (Figure 1). After three injections, no carryover could be observed, and the system was free of caffeine.

Reduction of pesticide carryover

A mixture of 13 pesticides with different polarities and chemistries was chosen to demonstrate the efficient carryover reduction for several compounds in a single analytical run. An undiluted pesticide standard was injected at a concentration of 100,000 µg/L. Because of the ultrahigh concentration, the sample was not introduced in the MS. Five blank injections of water followed for the determination of carryover (according to sequence table for caffeine). The calculation for the concentration of carryover of pesticides was as follows:

Absolute concentration of pesticide standard	Relative concentration of carryover
100,000 µg/L	1,000,000 ppm
100 µg/L	1,000 ppm
1 µg/L	10 ppm
0.1 µg/L	1 ppm

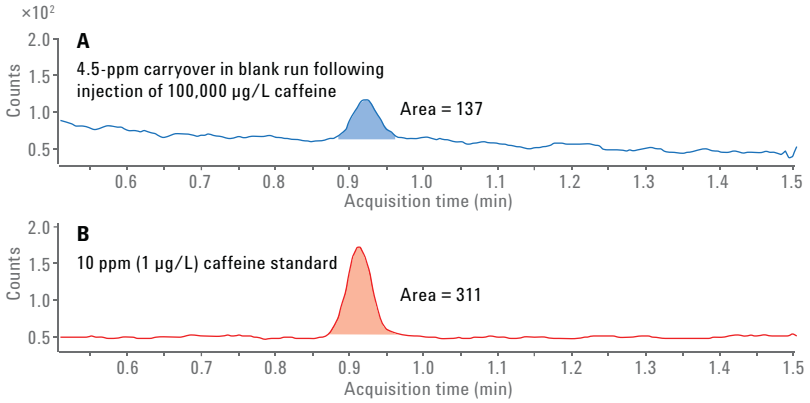

Figure 1. Blank injection after a 100-mg/L injection of caffeine standard (A). The carryover was determined to be 4.5 ppm. For comparison, (B) shows a 10-ppm (1-µg/L) caffeine standard.

For quantification of carryover, standards in the concentration range of 0.1 to 10 µg/L were prepared and analyzed. Different cleaning steps were tested with the multiwash function of the 1290 Infinity II multisampler. Finally, an efficient way to clean the needle and seat was to use isopropanol, acetonitrile, and water each containing 0.1 % formic acid (Table 3). More tips and tricks on how to optimize the washing procedure are described in an Agilent Technical Overview[2].

To ensure that the strong wash solvents (isopropanol and acetonitrile) are completely removed from the injection port (seat), the last washing step was to clean the seat for at least 18 seconds with the initial start conditions.

In different experiments, it was observed that carryover was mainly coming from the analytical column (Table 4). Therefore, a setup with alternating column regeneration was installed[3].

Two columns of the same chemistry were used whereby the sample was injected on one column, and a blank was injected on the second column.

The setup of two alternating columns required a valve installed in the column compartment for column switching. During the experiments, it was found that this valve could also be a source of carryover. To eliminate the valve as a carryover source, the two columns were exchanged manually after injection of the 100,000-µg/L pesticide sample, to investigate the amount of carryover caused solely by the 1290 Infinity II multisampler. Table 4 summarizes the results of the three different experimental setups.

Table 3. Multiwash method for carryover reduction of pesticides.

Step	Solvent	Wash time (s)	Seat backflush	Needle wash
1	Isopropanol + 0.1 % formic acid	5	Yes	Yes
2	Acetonitrile + 0.1 % formic acid	5	Yes	Yes
3	Water + 0.1 % formic acid	20	Yes	Yes

Table 4 demonstrates that the analytical column and the switching valve (for alternating column regeneration) were a source of carryover. These observations were also made by Dolan[1]. Up to 170 ppm carryover results from the column and up to 11 ppm results from the switching valve. The last column in Table 4 shows the carryover that originates exclusively from the 1290 Infinity II multisampler. The majority of pesticides had little detectable carryover (less than 9 ppm), when washed with the multiwash program described in Table 3. Pyraclostrobine and thiabendazole were the only compounds that were difficult to remove after three injections of blanks with a method optimized to cover a broad range of compounds.

Table 4. Results of carryover determination of pesticides after a 100,000-µg/L injection and three different experimental setups.

	System setup		
Compound	One column without switching valve (carryover of multisampler and column) (ppm)	Alternating column regeneration (carryover of multisampler and valve) (ppm)	Column changed by hand (carryover of multisampler) (ppm)
Imazapyr	66	30	19
Aminocarb	1.2	1.2	0.8
Thiabendazole	132	15	13
Metoxuron	36	3	3
Carbofuran	27	2.2	2.3
Imazalil	128	8	6.8
Metazachlor	73	2	2.5
Metosulam	98	5.6	5.2
Atrazine	56	2.1	2
Molinate	49	4.6	7.5
Diazinon	161	6.3	5.7
Pyraclostrobin	207	38	33
Dimethoate	15	0.8	0.6

Figure 2 shows another pesticide, diazinon, with a carryover in the first blank of 5.7 ppm. In the second blank, 1-ppm carryover was still observed, and in the third blank almost no carryover could be detected (S/N 2.8). The forth blank showed a signal to noise (S/N) of 1.5, and was below the limit of detection.

These data show that the multiwash feature of the 1290 Infinity II multisampler efficiently prevented and reduced carryover. To avoid carryover, it is important to ensure that all fittings are installed correctly, and that the final gradient hold is long enough. Alternating column regeneration is also an effective way to reduce carryover.

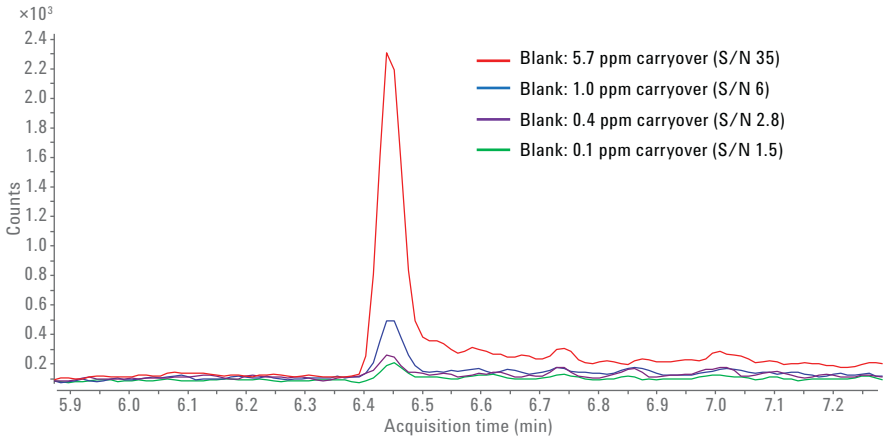

Figure 2. Calculated carryover of the first blank injection of diazinon with 5.7 ppm (5.7 µg/L) after a 100-mg/L pesticide standard injection. Carryover decreased with every following blank injection.

Conclusion

The multiwash function of the Agilent 1290 Infinity II multisampler is an optional feature to achieve ultralow carryover. The multiwash function cleans the outside of the needle and the needle seat by active backflushing with three different washing solvents. Using weak and strong wash solvents, contaminates can be reduced to enable higher sample throughput and eliminate false-positive results.

With a highly sensitive mass spectrometer, caffeine and a mixture of pesticides were injected at an extremely high concentration of 100,000 µg/L to determine carryover. Experiments with different system setups showed that the majority of carryover is caused by the analytical column and little carryover results from the column switching valve or the 1290 Infinity II multisampler. The calculated carryover after a 100,000-µg/L injection of caffeine for the 1290 Infinity II multisampler (excluding column and valve) was only 4.5 ppm, and for the majority of pesticides less than 9 ppm.

References

1. Dolan, J. W. Attacking Carryover Problems. *LCGC* **2001**, *Vol. 19, No. 10*.

2. Schuhn, B. Tips and Tricks for Achieving Near-Zero Carryover Using the Multiwash Function of the Agilent 1290 Infinity II Multisampler, *Agilent Technologies Application Note*, publication number 5991-6051EN, (**2015**).

3. Huesgen, A.G., Naegele, E., Automated alternating column regeneration on the Agilent 1290 Infinity LC, *Agilent Application Note*, publication number 5990-5069EN (**2009**).

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Published in the USA, October 1, 2015
5991-6246EN

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