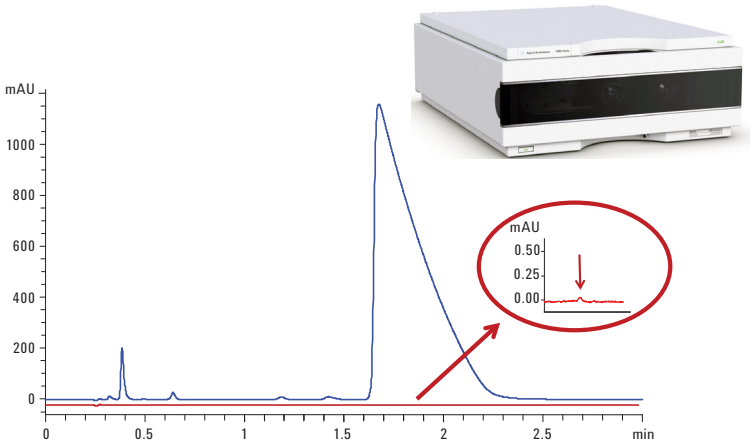
Near-zero carryover with flexible sample injection for high sensitivity trace analysis using the Agilent 1290 Infinity Flexible Cube

Technical Overview



Introduction

For modern high sensitivity liquid chromatography (LC) triple quadrupole mass spectrometry (MS) trace analysis, it is important to avoid any carryover of sample compounds from one sample injection to the next. To achieve the stringent requirement of near-zero carryover, it is necessary to develop software and hardware for specialized cleaning procedures of the LC autosampler. Modern ultrahigh performance LC (UHPLC) equipment enables fast analysis of samples, as well as support of these specialized cleaning procedures.

This work demonstrates the ability of the Agilent 1290 Infinity Flexible Cube, in conjunction with an Agilent 1290 Infinity LC System and an Agilent 6460 Triple Quadrupole LC/MS System, to achieve high sensitivity and near-zero carryover.

Agilent Technologies

Experimental

Equipment:

- Agilent 1290 Infinity LC System consisting of Agilent 1290 Infinity Binary Pump with integrated degasser, Agilent 1290 Infinity High Performance Autosampler with thermostat and Agilent 1290 Infinity Flexible Cube for near-zero carryover with variable sample amount injection and Agilent 1290 Infinity Thermostatted Column ompartment
- Agilent 6460 Triple Quadrupole LC/MS System
- Agilent MassHunter for data acquisition, qualitative and quantitative data analysis
- Column: Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm, 1.8 µm (p/n 827700-902)

LC method

Agilent 1290 Infinity Binary Pump

Flow:	0.5 mL/min
Isocratic:	65% Solvent A (Water + 0.1% TFA), 35% solvent B (AcN + 0.1% TFA)
Stop time:	2.5 min

Agilent 1290 Infinity Autosampler

Injection volume:	1 µL
Needle wash:	External needle wash, 15 sec with solvent A

Agilent 1290 Infinity Flexible Cube (Figure 1)

Time:	60 sec with solvent A in channel A1 at 4 mL/min

Agilent 1290 Infinity Thermostatted Column Compartment

Temperature:	30 °C

UV detection

Agilent 1290 Infinity Diode Array Detector

DAD:	257/4 nm, Ref. 360/16 nm, 10 mm path, 20 Hz data rate

Agilent 6460 Triple Quadrupole LC/MS System conditions

Source:	
Gas temperature:	300 °C
Drying gas:	10 L/min
Nebulizer:	35 psi
Sheath gas temperature:	400 °C
Sheath gas flow:	12 L/min
V_{cap}:	3000 V
Nozzle voltage:	0 V
Data acquisition:	MRM 1: 505.5 → 170.0, CE: 36 V, MRM 2: 505.5 → 201.2, CE: 20 V,
Fragmentor:	150 V
Delta EMV(+):	200 V

Sample:

Chlorhexidine:	Stock solution of 1 mg/mL in solvent A

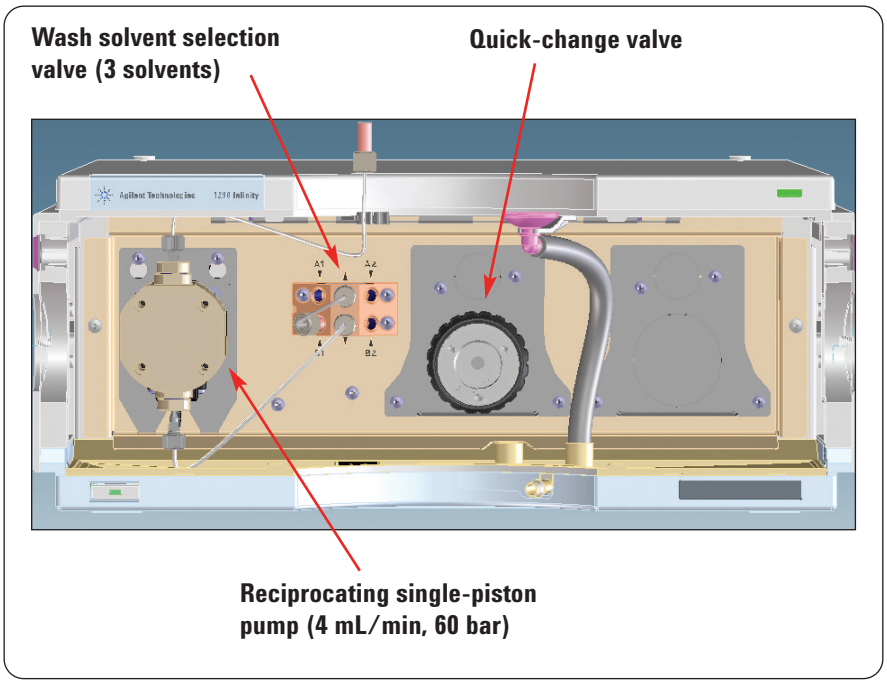


Figure 1
Agilent 1290 Infinity Flexible Cube LC module for needle seat backflush with variable injection volume, solvent selection, and valve.

Results and discussion

The Agilent 1290 infinity Flexible Cube module enables the Agilent 1290 Infinity Autosampler to work under near-zero carryover conditions supporting the flexible loop approach with the needle seat backflush mode (Figure 2). For the needle seat backflush (NSBF) mode, the pump in the Flexible Cube is connected directly to the injection valve in the autosampler. Solvents of different solution strengths can be chosen with the solvent selection valve in the Flexible Cube and used to wash the injection seat while the needle is kept above the seat.

The carryover with an included Agilent 1290 Infinity Flexible Cube in NSBF mode was initially measured for an Agilent 1290 Infinity LC System with Agilent 1290 Infinity DAD detection (Figure 3). Therefore, 3 µL of the chlorhexidine stock solution were injected and the area of the peak was compared to the area of the peak caused by carryover in a following blank injection. The determined carryover was 8 ppm. In this case no carryover has been detected for lower concentrations. If more chlorhexidine is injected, the measured carryover showed no increase and is therefore relatively lower compared to the injected amount.

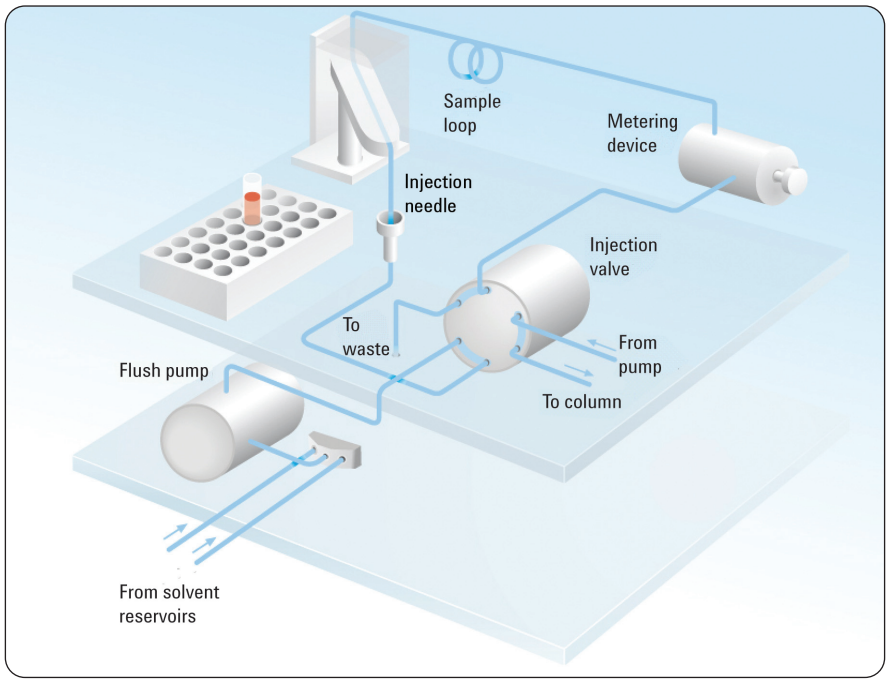


Figure 2
Agilent 1290 Infinity Flexible Cube LC module for elimination of carryover in variable loop injections by operation in seat backflush mode.

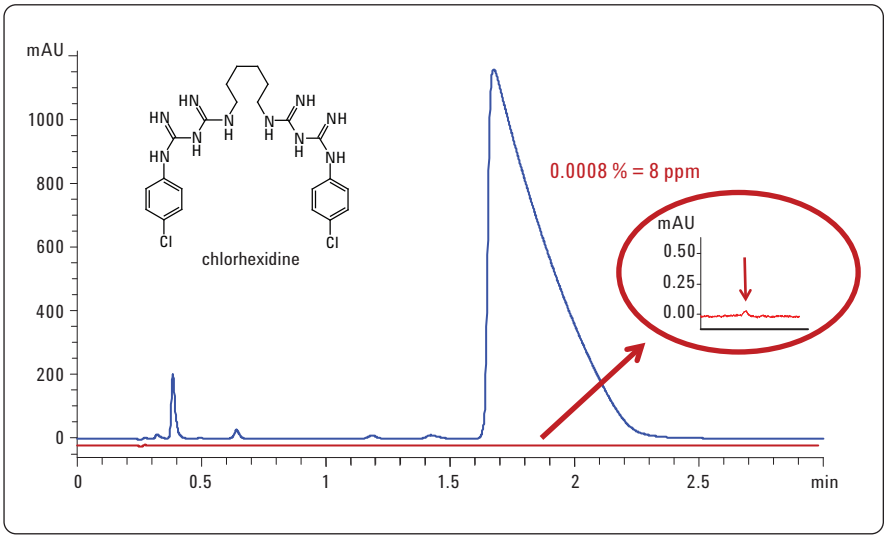


Figure 3
Experimental results for carryover reduction with the Agilent 1290 Infinity Flexible Cube in an Agilent 1290 Infinity LC System detected with an Agilent 1290 Infinity DAD (3 µL injection of 1 mg/mL (3 µg on-column) chlorhexidine followed by blank. The detected carryover was 8 ppm.

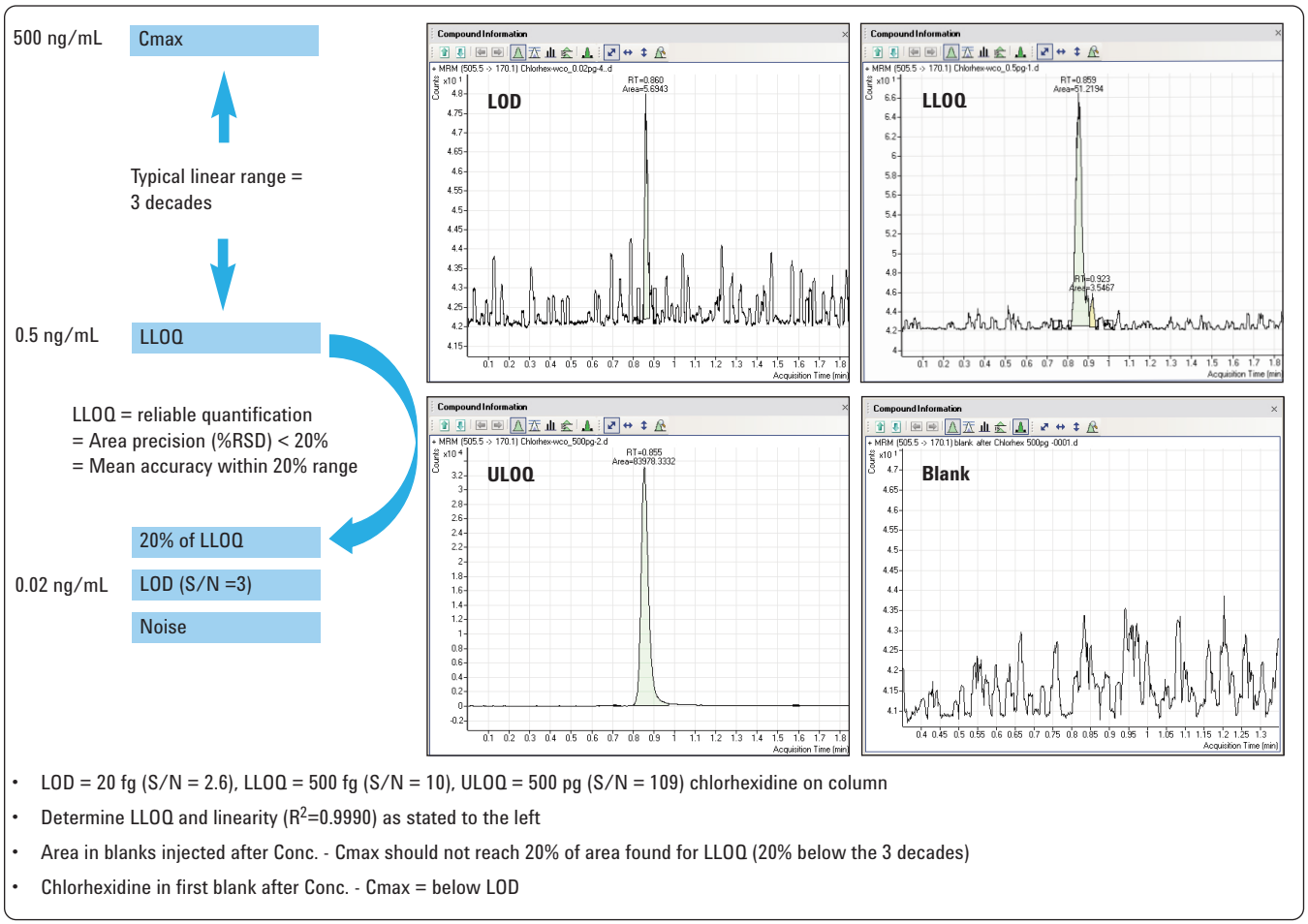


Figure 4
Procedure for determination of carryover in relation to the instrument sensitivity.

In order to determine carryover in relation to the instrument's sensitivity, a procedure for a highly sensitive triple quadrupole mass spectrometer was developed (Figure 4). The limit of detection (LOD, S/N = 3) and the lower limit of quantification (LLOQ, S/N=10) for a given compound (chlorhexidine) was determined. The instrument was calibrated for the usual analyte concentration (up to 500 µg/L = 1 µM) to the upper limit of quantification (ULOQ = 500 ng/mL), which is three decades above the LLOQ. A blank was injected after the injection of chlorhexidine at the ULOQ (500 pg on-column). The value of carryover should not exceed 20% of the LLOQ. The chlorhexidine peak was below the LOD (S/N = 1).

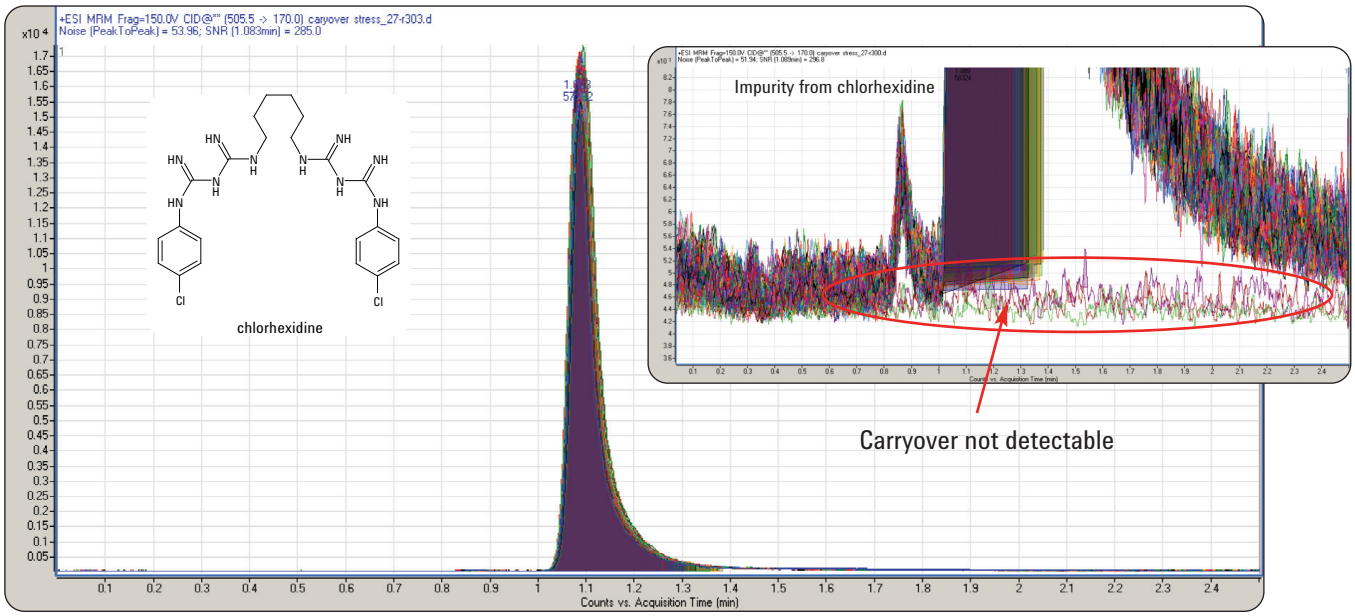


Figure 5
Robustness of carryover reduction – 600 injections of 1 ng chlorhexidine on-column and then three blanks.

The next point to test was the robustness to avoid carryover under standard operating conditions with a concentration slightly above the typical range. Therefore, about 600 injections of 1 ng chlorhexidine on-column were made. Then, three blanks were injected. There was no detectable carryover in all three blanks after the 600 repetitive injections of chlorhexidine (Figure 5). This demonstrated the robustness of the carryover reduction by the Agilent 1290 Infinity Flexible Cube, which avoids the accumulation of compounds even after hundreds of injections.

To stress the instrument, a concentration of 50,000 pg (50 µg/mL) (100 times the ULOQ) was injected and the "forced" carryover was determined (Figure 6). The carryover on-column was below 10 ppm.

Figure 6
Determination of forced carryover after an injection of 50 ng of chlorhexidine on column. Carryover < 10 ppm.

The performance gain of the Agilent 1290 Infinity LC System equipped with an Agilent 1290 infinity Flexible Cube was evaluated by comparing the results to those from an Agilent 1290 Infinity LC System without a Flexible Cube. For this comparison, an injection of 100 ng chlorhexidine was made on both system configurations. The systems had no column and a restriction capillary to measure the pure instrument carryover. The carryover from this highly concentrated injection in the following first blank was determined to be 55 ppm for the system not equipped with a Flexible Cube module. This was about five times more than in the system equipped with the Flexible Cube. More importantly, without Flexible Cube the carryover was also detectable in the second injection. The decreasing signal of the chlorhexidine carryover could be detected for a few more injections. With the Flexible Cube, no carryover is detectable within the second blank. This proves that the Flexible Cube prevents not only the carryover from highly concentrated sample injection, but also the permanent carryover typically resulting from this injection. (Table 1)

Without the Flexible Cube

Pure instrument carryover at 100 ng without column:

First blank:	0.006 %, (55 ppm, 5.5 pg)
Second blank:	0.0015 %, (6 ppm, 1.5 pg)

Column replaced by restriction capillary.

With the Flexible Cube no carryover is detectable within the second blank!

Table 1
Carryover behavior without Agilent 1290 Infinity Flexible Cube.

Conclusion

This work demonstrates the use of the Agilent 1290 Infinity LC System in combination with the Agilent 1290 Infinity Flexible Cube module for carryover reduction in high sensitivity triple quadruploe LC/MS applications. A method for the determination of carryover in relation to the instrument sensitivity was developed. It was demonstrated that the measured instrument carryover within the normally used concentration range is zero. It was also demonstrated that even with a 100 times more excess concentration, the forced carryover is near zero and can be neglected.

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