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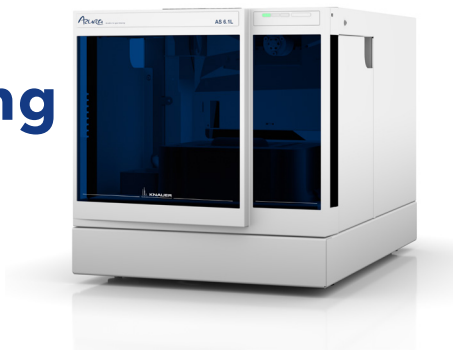
Investigation of carryover under consideration of different washing solvents and volumes



Svea Stephan, Till Myrach, Carsten Losch, Kate Monks; applications@knauer.net
KNAUER Wissenschaftliche Geräte GmbH, Hegauer Weg 38, 14163 Berlin; www.knauer.net.

SUMMARY

In this study it was investigated to what extent the carryover correlates with the number of subsequent washing steps. Both the wash volume and the properties of the wash solutions have a significant effect on sample carryover. Using three washing steps (750 µL total volume) sample carryover was reduced to less than 0.003 % for both analytes, caffeine and chlorhexidine. The sample carryover can be further reduced by a factor of 10 if the extended wash function is used. With the extended wash function, two different wash solutions are used. The first is usually a strong organic solvent, that should remove all remaining sample residues. The second wash solution is subsequently used to completely rinse the organic wash solution out of the fluidic system. The eluent with the lowest elution strength is particularly suitable for this purpose. In a sample application, using the described extended wash, a lower carryover of only 0.0003 % was achieved. This low value makes the procedure especially advantageous for trace analyses using highly sensitive MS/MS or electrochemical detectors.

INTRODUCTION

Carryover is the appearance of a small sample peak when injecting a blank after the sample [1]. It is caused by sample residues which remained in the system after the preceding injection. One significant factor for carryover is the injection mode used. In microliter pickup mode only the volume of sample that should be injected is aspirated. When using the full loop mode on the contrary, not only the loop volume but also an additional volume of up to three times the loop volume is aspirated. This overfilling is done to ensure that the loop is completely filled with sample guaranteeing maximum reproducibility. Furthermore, the carryover is also influenced by the sample itself as its adhesive properties define how strong it sticks to the surface area. To prevent a carryover between two analyses a thorough wash procedure is necessary to remove sample residues in the injection system. Especially with very sensitive detection methods minimizing carryover is necessary to avoid false-positive results. In this study the carryover of the autosampler KNAUER AZURA AS 6.1L was determined and an optimized washing procedure was developed.

Additional Information

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RESULTS

One of the two main reasons for carryover are small cavities in the system. A tiny amount of sample in these reservoirs is diluted over time and the carryover decreases with every injection. This carryover can easily be determined with caffeine as analyte. The carryover of the AZURA HPLC system was analyzed for different injection modes and three different wash steps **(Tab. 1)**.

Tab. 1 Carryover of caffeine for different injection modes and with 1, 2 and 3 wash steps (250 µL each)

Carryover [%]	Microliter pickup	Partial loop	Full loop
1 wash step	0.0000	0.0196	0.0000
2 wash steps	0.0000	0.0029	0.0000
3 wash steps	0.0000	0.0024	0.0000

Both in full loop and in microliter pickup injection mode no carryover is detectable even with only one wash step. In partial loop mode a carryover occurs but already two wash steps reduce it to under 0.005 %. The second main reason for carryover is a chemical adsorption of sample to the wetted surface of sample loop, needle, tubings and valves of the injection system. It can be determined with a sticky substance like chlorhexidine. The determination of the carryover of chlorhexidine was combined with the development of an optimized washing procedure for the AS 6.1L injection system **(Tab. 2)**.

Tab. 2 Carryover of chlorhexidine for different injection modes with 1, 2 and 3 wash steps (250 µL each)

Carryover [%]	Microliter pickup	Partial loop	Full loop
1 wash step	0.0648	0.0171	0.0019
2 wash steps	0.0041	0.0020	0.0006
3 wash steps	0.0017	0.0019	0.0006

In all three injection modes two wash steps lead to a carryover of less than 0.005 %. Since the highest value was observed with microliter pickup, this mode was chosen to optimize the wash procedure. For this procedure two different wash solutions were used. The first wash step with isopropanol should remove chlorhexidine, while the subsequent step with mobile phase is intended to remove the isopropanol again. Starting with a combination of 750 µL isopropanol and 750 µL eluent the volumes were increased in steps of 250 µL up to 1500 µL. The results for carryover are shown in **Tab.3**.

Tab. 3 Carryover of chlorhexidine with different volumes of isopropanol used in the first washing step and eluent used in the second washing step

Wash step 1 Isopropanol [µL]	Wash step 2 Eluent [µL]	Carryover [%]
750	750	0,0028
1000	1000	0,0031
1250	1250	0,0022
1500	1500	0,0003
1500	750	0,0016

It is noticeable that there is no significant difference between 2 x 750 µL and 2 x 1000 µL. From 1250 µL the carry over decreases significantly and at 1500 µL almost no carry over is detectable. A combination of 1500 µL isopropanol followed by 1500 µL mobile phase led to a carryover of 0.0003 %. **Fig. 1** shows the blanks before and after injection of the chlorhexidine standard with 2000 µg/mL compared to the concentration of 10 µg/mL. Whether the washing liquid isopropanol has to be adapted to the analyte should be investigated in each individual case.

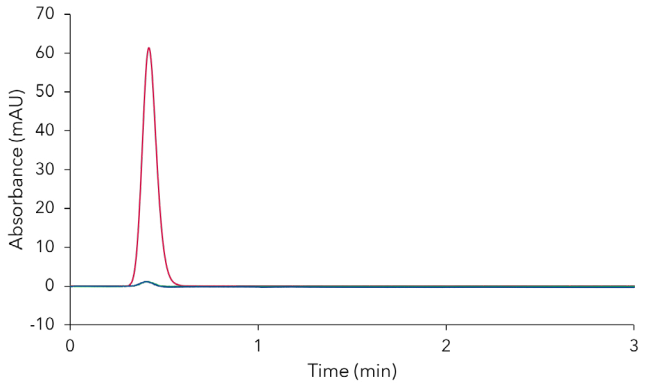

Fig. 1 Injection of chlorhexidine standard 10 µg/mL (red), Blank 1 (blue) before and Blank 2 (green) after the injection injection of the chlorhexidine standard with 2000 µg/mL, in between rinsing steps with 1500 µL isopropanol and 1500 µL eluent (microliter pickup mode).

CONCLUSION

The carryover of the AZURA AS 6.1L resulting from small cavities in the system can be reduced to under 0.003% by including two wash steps. For system setups with sensitive detectors or while working with sticky substances the use of an optimized wash procedure can be beneficial. The wash procedure, which combines 1500 µL isopropanol and 1500 µL mobile phase, reduces the carryover to 0.0003% in partial loop injection mode. The carryover in full loop and microliter pickup mode is even lower. Since the value of sample carryover is both substance and method-specific, any method development should include an investigation of sample carryover. This investigation should consist of not only the chromatographic method but also the sample preparation.

REFERENCES

[1] Zeng, W., Musson, D. G., Fisher, A. L. and Wang, A. Q. (2006), A new approach for evaluating carryover and its influence on quantitation in high-performance liquid chromatography and tandem mass spectrometry assay. Rapid Commun. Mass Spectrom., 20: 635-640.

MATERIALS AND METHODS

Tab. 4 System configuration

Instrument	Description	Article No.
Pump	AZURA P6.1L HPG	APH35EA
Autosampler	AZURA AS 6.1L	AAA00AA
Detector	AZURA DAD 6.1L	ADC11
Flow Cell	LightGuide UV Flow Cell, 10 mm, 2 µL	AMC19XA
Thermostat	AZURA CT 2.1	A05852
Restriction capillary	ID 0.18 mm, L 15 m	A3313
Software	ClarityChrom 8.1	A1670

Analyte	CAS	Manufacturer
Chlorhexidine	55-56-1	Sigma Aldrich
Caffeine	58-08-2	Sigma Aldrich

Tab. 5 Method parameters for the determination of the carryover

Parameter	
Flowrate	1 mL/min
Eluent	water
Detection Wavelength	272 nm (caffeine)
257 nm (chlorhexidine)	0
Data Rate	10 Hz
Time constant	0.1 s
Injection volume	10 µL (microliter pickup, partial loop)
100 µL (full loop)	
Buffer tubing	500 µL
Sample loop	100 µL
Needle tubing SSt	15 µL
Syringe	250 µL

Tab. 6 Injection scheme for determination of carryover

No.	Sample	Explanation
1	Blank 1	Determination of the solvent peak
2	Low conc. (10 µg/µL)	Reference sample, for calculation of the carryover
3	high conc. (2000 µg/µL)	Highly concentrated sample, exceeding the linear range of the detector
4	Blank 2	Determination of carryover
5	Blank 3	
6	Blank 4	

Tab. 7 Calculation of carryover

$$\text{Carryover} = \frac{A_{Blank2} - A_{Blank1}}{A_{low} * (C_{high} / C_{low})} * 100$$

A_{Blank1}:	Area of first blank injection
A_{Blank2}:	Area of second blank injection
A_{low}:	Area of the standard with low concentration
C_{high}:	Concentration of sample with high concentration
C_{low}	Concentration of sample with low concentration

