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Impact of Autosampler Design on Carryover Performance During Method Migration

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PURPOSE

Method migration or moving methods from one system to another can be challenging due to differences in design and method settings across systems. Learning about the similarities and differences between system designs, and how these can impact method migration will allow users to move methods between systems with a higher level of confidence.

For example, the differences in autosampler characteristics can impact carryover leading to failure to meet system suitability requirements or to inaccurate quantitative results. To reduce variability across systems, understanding and optimizing needle wash settings is essential in method migration and controlling carryover.

OBJECTIVE

In this study, the impact of instrument design and needle wash settings are evaluated for their impact on carryover, including both volumetric and absorptive.

METHODS

Caffeine Carryover

Caffeine Challenge Solution: 4 mg/ml in 90:10 Water: Acetonitrile
Calibration Curve (µg/ml): 0.01, 0.02, 0.04, 0.12,and 0.4
Blank: 90:10 Water: Acetonitrile
Column: CORTECS™ C18, 2.7 µm, 3 mm × 100 mm (p/n: 186007372)
Column Temp: 40 °C, Sample Temp: 10 °C, Injection Volume: 10 µl
Flow Rate: 1.8 ml/min
Needle Wash Solvent: Varies, Needle Wash time: Varies
Mobile Phase A: Water, Mobile Phase B: Acetonitrile
Isocratic: 90:10 mobile phase A: mobile Phase B, Run Time: 4 minutes
PDA Wavelength: 273 nm

Chlorhexidine Carryover

Chlorhexidine Standard Solution: 1mg/ml Chlorhexidine in 0.1% TFA in water
Calibration Curve (µg/ml): 0.012, 0.048, 0.12, 1, and 10
Blank: 90:10 Water: Acetonitrile
Column: CORTECS™ C18, 2.7 µm, 3 mm × 100 mm (p/n: 186007372)
Column Temp: 50 °C, Sample Temp: Room temp, Injection Volume: 5 µl
Flow Rate: 1 ml/min
Needle Wash Solvent: Varies, Needle Wash time: varies
Mobile Phase A: 0.1%TFA in Water, Mobile Phase B: 0.1% TFA in Acetonitrile
Isocratic 67:33 mobile phase A: mobile Phase B, Run Time: 10 minutes
PDA Wavelength: 257 nm

USP Monograph for Chlorhexidine (adapted)

Sample: 1.14 mg/ml Chlorhexidine
Diluted Sample: 11.4 ug/ml Chlorhexidine
Blank: 80:20 Water: Acetonitrile +0.1%TFA
Detector Mode: Single Wavelength, Sampling Rate: 2 pts/sec
Channel A Wavelength: 254 nm
Injection Volume: 10.0 µL, Sample Temperature: 8.0 °C
Column Temperature: 30.0 °C
Flow Rate: 1ml/min
Column: XSelect™ HSS C18 SB, 250 x 4.6 mm 3.5 µm (p/n: 186004751)
Mobile Phase A: 80:20 Water: Acetonitrile + 0.1%TFA
Mobile Phase B: 10:90 Water: Acetonitrile + 0.1%TFA
Wash Solvent: 50:50 Water: Acetonitrile

Time	%A	%B	Curve
0.00	100	0	6
2.00	100	0	6
32.00	80	20	6
37.00	80	20	6
47.00	70	30	6
54.00	70	30	6
55.00	100	0	6
65.00	100	0	6

RESULTS

To control for carryover, most liquid chromatography (LC) systems have mechanisms to wash the needle or injector either **pre- or post-aspiration** of the sample or **pre- or post-injection** of the sample. For example, the ACQUITY Arc™ System has a flow through needle and allows for washing of the needle pre- or post- injection with a wash solvent. In the pre-injection wash mode, the exterior of the needle is washed but it is not in the seal or under pressure during washing. Alternatively in the post-inject mode the exterior of the needle is washed but the needle is in the seal and under high pressure. In this study, two analytes were tested to evaluate the impact of the injector design, wash mechanism, and wash solvent on both absorptive and volumetric carryover.

To evaluate **volumetric carryover,** or carryover due to an analyte getting trapped in the system, a challenge sample of caffeine was chosen. Results for the ACQUITY Arc System (Figure 1) show that the highest carryover was observed with no washing of the needle, as expected. By adding both pre- and post-injection washing, carryover was significantly reduced. The lowest volumetric carryover was observed with both settings suggesting washing the needle pre-injection/post-aspiration and post-injection both remove the analyte in the flow path. The needle wash solvent composition did not appear to have a significant impact on carryover further suggesting volumetric carryover (data not shown). It is important to note that all values were well below the specification of <0.002% [1].

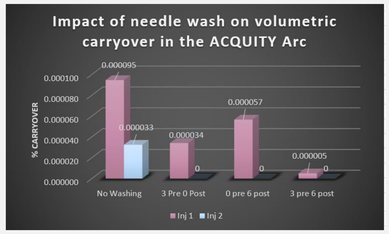

Figure 1. Impact of needle washing mode on volumetric carryover on ACQUITY Arc System for four different wash modes. Default (0 sec pre and 6 sec post) and three other wash settings were evaluated. Highest carryover observed with no needle washing.

The same study was performed on a system (System X) that uses a pre-injection/post-aspiration needle washing mechanism. Both systems were evaluated with their default settings (3 seconds pre for System X and 6 seconds post for the ACQUITY Arc System). To evaluate the impact of pre-injection washing on the AQCUITY Arc System a 3 seconds pre-injection was added to the default. The latter conditions produced the lowest carryover (Figure 2). Furthermore, the ACQUITY Arc System showed no carryover on the second post blank injection, while System X continued to have measurable carryover. These results suggest that the ACQUITY Arc System successfully removes volumetric carryover after a single blank. All values were well within specifications for both systems (System X - < 0.003% (30 ppm)[3], and ACQUITY Arc - <0.002%[1]).

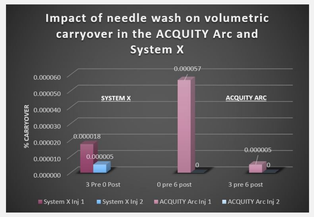

Figure 2. Comparison of ACQUITY Arc System and System X for impact of needle washing modes on volumetric carryover.

While volumetric carryover is often a concern, carryover from **absorption or an analyte that "sticks" to the flow path surface** can be more challenging to remove. For this study, a short method using chlorhexidine was analyzed. Testing on the ACQUITY Arc System shows the impact of wash solvent on carryover, with the lower organic composition resulting in higher carryover as compared to higher organic wash solvents. The mode of washing the needle did not appear to have as large of an impact on absorptive carryover indicating this type of carryover is better controlled by altering the needle wash solvent.

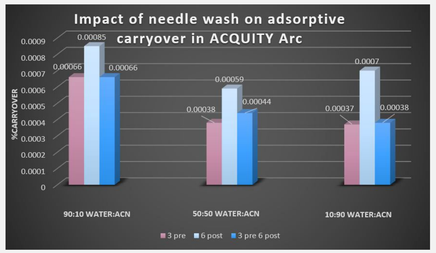

Figure 3. Impact of needle washing mode and needle wash composition on absorptive carryover for ACQUITY Arc.

Although carryover is a challenge for many methods, many routine assays or monographs have conditions that may invertedly increase the likelihood of carryover. To assess the impact of more typical method conditions, a method based on the **USP monograph for chlorhexidine HCl organic impurities** [2] was evaluated.

For this analysis, the method was tested on both the ACQUITY Arc System and a system with pre-injection/post-aspiration needle washing only (System X). Both systems were tested using the default configuration and 50:50 water:acetonitrile wash solvent. Carryover was calculated after 6 injections of the sample followed by 3 post blank injections. For the ACQUITY Arc System there was no detectable carryover. In contrast, System X had measurable carryover (Figure 5). Furthermore, the carryover did not appear to decrease with subsequent post injection blanks.

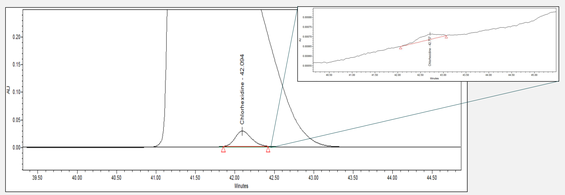

Figure 4 Overlay of the chlorhexidine HCl sample at 1.4 mg/ml, diluted sample (11.4 ug/mL) and post blank 1 from System X under the USP monograph organic impurities method conditions. The post blank carryover was hard to see on the same scale so it has been added in a zoomed in image.

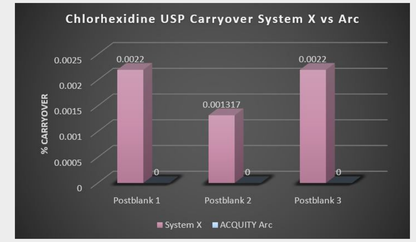

Figure 5 Comparison of carryover for ACQUITY Arc System and System X for method based on USP monograph for chlorhexidine. The ACQUITY Arc System showed no detectable carryover while System X had measurable carryover, which did not decrease with subsequent post-blank injections. Both systems were run with their system default wash.

CONCLUSIONS

Carryover can be a challenge for many assays, as it can impact quantitation and system suitability. To control for carryover in any method, it is critical to understand both the type of carryover as well as the autosampler design and tools available to control carryover.

In this study, **volumetric carryover** was found to be best controlled by needle wash settings, including duration of washing and sequence of washing in the injection cycle (pre-or post-injection and pre- or post-aspiration). For the ACQUITY Arc System using both a 3 second pre-injection and a 6 second post-injection wash was found to produce the lowest volumetric carryover.

Alternatively, **adsorptive carryover** was found to be controlled predominantly by the needle wash solvent, where stronger needle washes were found to generally produce the lowest carryover. Furthermore, for a challenging HPLC method, the injector design was critical to reduce carryover. In this example, the ACQUITY Arc System using post-injection/post-aspiration washing produced much lower carryover than a system that only performed pre-injection/post aspiration washing.

REFERENCES

1. ACQUITY Arc System Guide, Document number 715004747 Version 04
2. United States Pharmacopeia (2021). *USP Monographs, Chlorhexidine Hydrochloride.* USP-NF. Rockville, MD:USP. DOI: https://doi.org/10.31003/USPNF_M15650_03_01
3. Agilent 1260 Infinity II Multisampler Data Sheet https://www.hpst.cz/sites/default/files/download/2021/11/5991-7092en_1260_ii_multisampler_datasheet.pdf

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