Impact of Instrument Design on Absorptive Carryover

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Introduction

Carryover is a common problem encountered when running methods on high pressure liquid chromatographic (HPLC) systems, particularly for quantitative analysis and/or sensitivity analyses. For many methods, absorptive carryover is particularly problematic as it is caused by the analyte sticking to the surface of the system flow path. To reduce or eliminate absorptive carryover, most modern HPLC systems incorporate some type of a needle wash during the injection cycle. The choice of needle wash solvent is critical since it should be of a suitable composition to dissolve any compound of interest which can then be flushed to waste. Depending on the autosampler design, needle washing can occur prior to and/or after injection, or prior to and/or after sample aspiration. The mechanism under which the needle is washed and the timing and duration that this step can have a significant impact on carryover. In this study, absorptive carryover is investigated on five HPLC systems, and the impact of injector design is evaluated.

Method

Method Conditions	
Wavelength	254 nm
Column(s)	XSelect™ HSS C18 SB, 250 x 4.6 mm 3.5 µm (p/n: 186004751)
Column Temp	30 ºC
Sample Temp	8 ºC
Injection Volume	10 µL
Flow Rate	1 mL/min
Mobile Phase A	80:20 0.1% TFA in Water:0.1% TFA in Acetonitrile
Mobile Phase B	10:90 0.1% TFA in Water:0.1% TFA in Acetonitrile
Needle Wash	50:50 Acetonitrile:Water
Seal Wash	10:90 Acetonitrile:Water

Instruments
Arc HPLC System™
System V
System Y
System Z

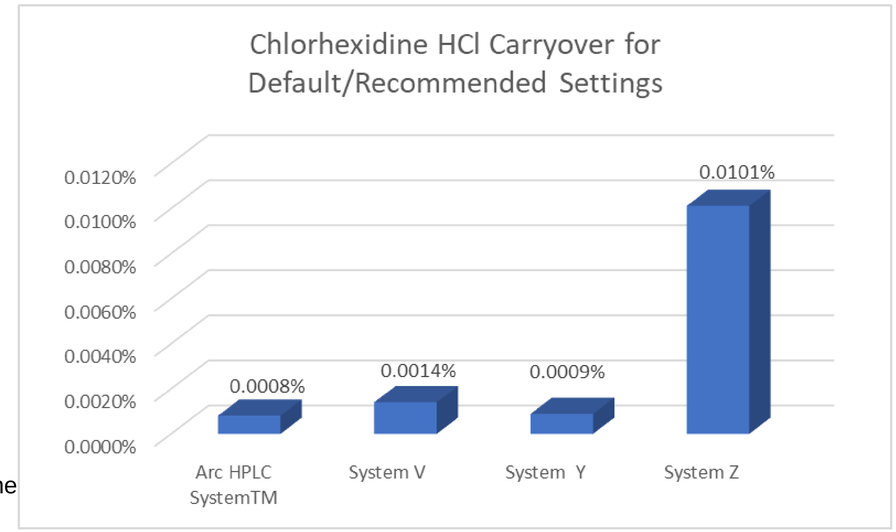
This method was adapted from the USP monograph for Chlorhexidine organic impurities. A TUV detector has been used with all instruments.

Results and Discussion

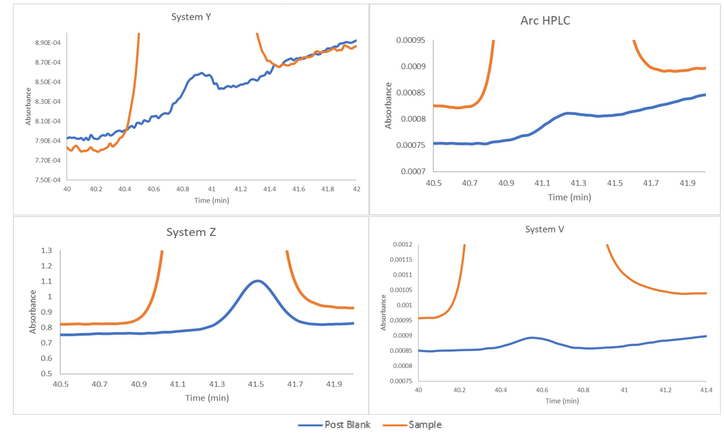
Gradient Table		
Time	A%	B%
0.00	100.0	0.0
2.00	100.0	0.0
32.00	80.0	20.0
37.00	80.0	20.0
47.00	70.0	30.0
54.00	70.0	30.0
55.00	100.0	0.0
65.00	100.0	0.0

Sample List		
Sample	Purpose	Replicate
Mobile Phase A	Blank	2
Diluted Sample Solution	Standard for quantitation (11.4 µg/mL)	6
Sample Solution	Sample (1.14 mg/mL)	6
Post Blank (Mobile Phase A)	Carryover (any chlorhexidine present reported as % carryover)	1
Mobile Phase A	Blank	2

Instrument	Recommended/Default Needle Wash Settings
Arc HPLC System™	After injection for 6 seconds
System V	Before aspiration for 13 seconds
System Y	Before and after aspiration by dipping in wash solution
System Z	No needle washing



System Z does not include a needle wash step in its recommended settings. Without a needle wash step the carryover is the highest. Although System Y, Arc HPLC System™ and System V show negligible carryover in real analysis, among them System V shows higher carryover. System Y performs a needle wash step both before and after sample aspiration by dipping the needle in wash solution and Arc HPLC System™ includes a needle wash step after injection which lasts 6 seconds.



Conclusion

The lack of a needle washing step in the default/recommended settings of the instrument leads to significant carryover. Implementing a needle wash step significantly reduces the carryover and this reduction in the carryover depends on the mechanism under which the needle is washed and the length of the needle washing step. Overall, the data collected in this study emphasizes the importance of washing the needle and the selection of appropriate needle wash parameters to control absorptive carryover.

Reference

1-Dlugasch, A.; Simeone, J.; McConville, P. Alliance Carryover Performance Part 1: Carryover Improvement Achieved Through Instrument Design Changes for the Alliance HPLC System. Waters Application Note, 720006386EN, 2018.

2-USP, Chlorhexidine Hydrochloride, United States Pharmacopeia and National Formulary (USP43-NF38 – 952), 2022 (DocId: GUID-933701DC-1DF7-435F-A968-80C401EA550E_3_en-US).