NEEDLE WASH MECHANISM AND ITS IMPACT ON HILIC ANALYSIS

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INTRODUCTION

Most regulated laboratories tend to have multiple HPLC systems, which may have design differences available to help optimize their workflows. Because of these design differences, methods are migrated from system to system to ensure comparable results are generated and the workflow is not impacted.

One such type of method available in these regulated laboratories is the Hydrophilic Interaction Liquid Chromatography (HILIC) method. HILIC methods present migration issues, especially for users who are not as familiar with its unique characteristics and dealing with design differences from one HPLC system to another.

While attempting to migrate the USP Cetirizine Hydrochloride Organic Impurities method between two HPLC systems, system suitability could not be met due to a split peak being observed in the system suitability solution sample on one of the systems. Because the presence of excess water in a HILIC method can pose chromatographic issues, investigative work was performed to determine if excess water was the root cause of the split peaks observed.

This study was performed to explore how the difference in autosampler design between the two HPLC systems can affect the results of a HILIC method analysis when varying wash solvent compositions are utilized.

METHODS

Cetirizine Hydrochloride USP Monograph Organic Impurities Conditions[1]:

LC System:	Alliance™ iS HPLC System System X HPLC System
Detection:	Dual Wavelength UV Detector, 230 nm @ 10 points/second Variable Wavelength Detector (VWD), 230 nm @ 10Hz
Column:	XBridge™ BEH™ HILIC Column, 130Å, 5-µm, 4.6mm × 250mm (p/n 186004454)
Column Temp.:	25 °C
Sample Temp.:	10 °C
Injection Volume:	10 µL
Flow Rate:	1.0 mL/min
Mobile Phase:	Acetonitrile (ACN):Water:1M sulfuric acid (93:6.6:0.4)
Needle Wash:	ACN:Water (93:7) ACN:Water (80:20) ACN:Water (60:40) ACN:Water (40:60) ACN:Water (20:80) ACN:Water (7:93)
Method:	*Organic Impurities: Isocratic 18-minute method*

System Suitability Requirements:

- Tailing is NMT 2.0 for cetirizine
- Resolution is NLT 2.0 between cetirizine and cetirizine related compound A

Sample:
System Suitability Solution consists of 4.0 µg/mL cetirizine hydrochloride and 4.0 µg/mL cetirizine related compound A in mobile phase.

Note: Needle wash line was flushed for 6 cycles before each analysis to ensure prior wash solvent was replaced by the new wash.

RESULTS & DISCUSSION

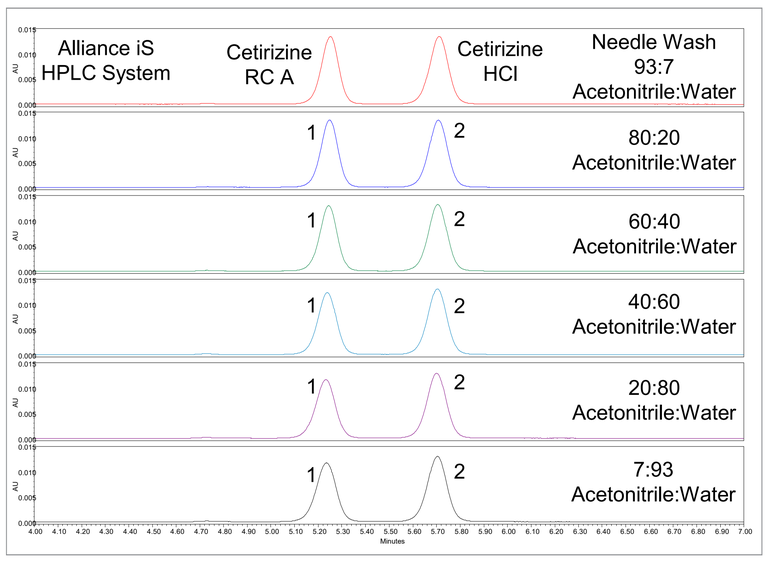

Figure 1. Impact of Needle Wash on HILIC USP Monograph on Alliance iS HPLC System.
***Peak 1:** Cetirizine RC A **Peak 2:** Cetirizine HCl*

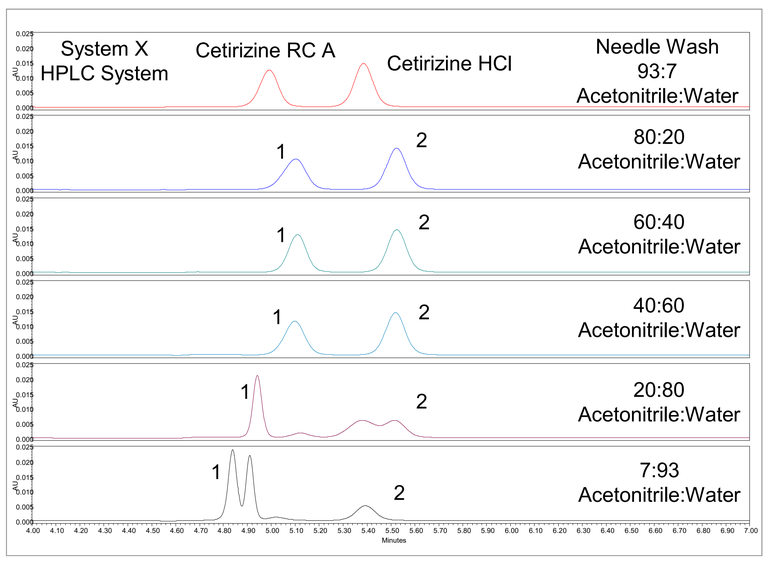

Figure 2. Impact of Needle Wash on HILIC USP Monograph on System X HPLC System.
***Peak 1:** Cetirizine RC A **Peak 2:** Cetirizine HCl*

For this study, 6 different needle wash solutions were prepared to be used for analysis. Two HPLC systems, Alliance iS HPLC System and System X HPLC System, were chosen to perform this study to help illustrate how a difference in needle wash design and mechanism may play a role in producing unacceptable chromatography.

Cetirizine Hydrochloride System Suitability Solution Tailing Factor (NMT 2.0)		
Needle Wash	**HPLC System**	
	Alliance iS HPLC System	**System X HPLC System**
ACN:Water (93:7)	1.0	1.0
ACN:Water (80:20)	1.0	1.0
ACN:Water (60:40)	1.0	1.0
ACN:Water (40:60)	1.0	1.0
ACN:Water (20:80)	1.0	Could not Process
ACN:Water (7:93)	1.0	Could not Process

Table 1. Tailing Factor results for the various wash needle compositions. Results are an average of 3 replicate injections

Cetirizine Hydrochloride System Suitability Solution Resolution (NLT 2.0)		
Needle Wash	**HPLC System**	
	Alliance iS HPLC System	**System X HPLC System**
ACN, water (93:7)	3.2	2.6
ACN, water (80:20)	3.2	2.3
ACN, water (60:40)	3.2	2.8
ACN, water (40:60)	3.1	2.5
ACN, water (20:80)	3.0	Could not Process
ACN, water (7:93)	2.9	Could not Process

Table 2. Resolution results for the various needle wash compositions. Results are an average of 3 replicate injections

Figure 1 displays the System Suitability chromatograms obtained using the Alliance iS HPLC System for all six needle wash compositions examined. Regardless of the wash composition used, the chromatography is visually acceptable and meets system suitability requirements as seen in Tables 1 and 2.

RESULTS & DISCUSSION

Figure 2 displays the System Suitability chromatograms obtained using the System X HPLC for all needle wash compositions examined. There is clearly significant peak splitting when a more aqueous needle wash is used. Because water is the strong solvent in HILIC chromatography, one can extrapolate that the peak splitting is due to residual wash solvent being left on the needle surface, which is subsequently injected with the next sample. The System X HPLC system meets system suitability requirements while using needle wash compositions of 93:7, 80:20, 60:40, and 40:60 Acetonitrile:Water as seen in Tables 1 and 2. However, System X HPLC system is unable to meet system suitability requirement while utilising 20:80 and 7:93 Acetonitrile:Water.

With System X HPLC system showing chromatographic issues, which are not observed on the Alliance iS HPLC System, a closer look at the needle wash mechanism on both systems may help explain the issues observed. On the Alliance iS system, the needle is washed before and after the injection at the same location. The wash is introduced from the bottom and top of the injection housing and is aspirated out through the puncture needle mitigating wash solvent getting introduced into the injection port (Figure 3).

The wash sequence on System X HPLC system involves the needle being dipped in the wash solvent at a wash station before being sent to the injection port to commence injection. This dipping motion allows for excess wash to linger on the needle and travel to the injection station to be injected along the sample. This could explain the excess water present in the sample at injection and causing the split peaks observed.

It is common for needle wash compositions to be relatively strong to minimize method carryover. In this example, a strong needle wash can cause chromatographic issues, specifically peak splitting, depending on the design of the autosampler washing mechanism. When peak splitting is seen in chromatography, common sources are strong solvent effects, column/stationary phase problems, clogs somewhere in the system, etc., and many chromatographers may not consider the impact of the needle wash composition and washing mechanism as a potential cause of the observed peak splitting.

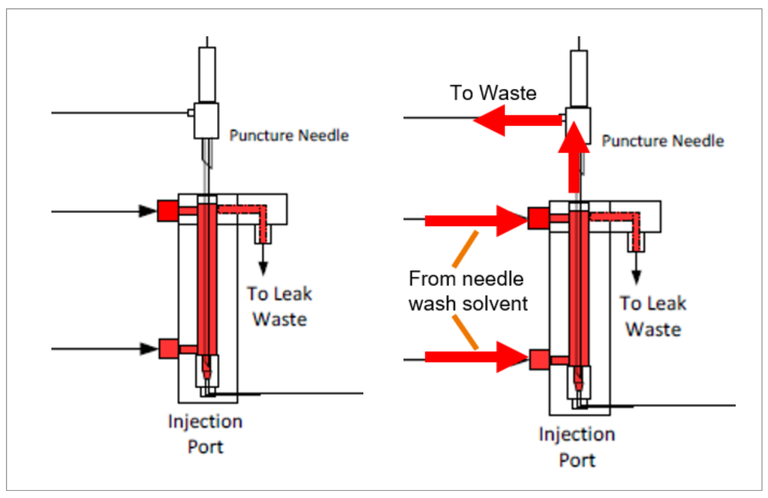

Figure 3. Fluidic diagram for Alliance iS HPLC System injection station.

CONCLUSION

- **Migration of HILIC methods can pose challenges due to unfamiliarity of technique and considerations for needle washes.**
- **The Alliance iS HPLC System met system suitability requirements for Cetirizine Hydrochloride USP Organic Impurities Monograph, a HILIC method, regardless of needle wash composition.**
- **For System X HPLC system, needle wash composition was found to impact the peak shape and/or chromatography, potentially due to the mechanism of needle washing.**

References
1. Monograph: USP. Cetirizine Hydrochloride. In: USP-NF. Rockville, MD: USP; 01 Sep 2021. DOI: https://doi.usp.org/USPNF/USPNF_M2902_06_01.html