Considerations for HILIC Method Migration

Elom Pedanou, Kevin Witter, Lise Gauthier and Paula Hong

Waters Corporation, Milford, Massachusetts, USA

INTRODUCTION

Hydrophilic Interaction Chromatography (HILIC) methods can be found in regulated laboratories that have different chromatographic systems present for analysis. HILIC methods can be migrated from system to system but a lack of knowledge about the chemistry of HILIC separations and understanding of the instrument design may lead to challenges when migrating the methods across systems. Given their unique characteristics, properly setting up a system including washes, for HILIC separations can be complex. One issue is the use of an incorrect needle wash solvent composition on the system which may stem from monographs not specifying wash solvent compositions.

In this presentation, the USP Cetirizine Hydrochloride Assay and Organic Impurities method was used to demonstrate the impact of various needle wash solvents on a HILIC chromatographic separation. Due to the lack of guidance towards needle wash composition, a strong aqueous solution would be the preferred needle wash to dissolve trace amounts of the compounds in the analysis to help mitigate carryover. However, this may create some issues such as peak splitting from excess water being introduced to the sample matrix, due to the design of the autosampler, thus failing to meet system suitability requirements. This can lead to time being wasted looking into root causes such as column and sample preparation while the needle wash may be overlooked.

METHOD

Cetirizine Hydrochloride USP Monograph Organic Impurities Conditions[1]:

Mobile Phase: Acetonitrile, water, and 1 M sulfuric acid (93:6.6:0.4)
Detector: UV 230 nm
Column: XBridge™ BEH™ HILIC Column, 130Å, 5-µm, 4.6mm × 250mm P/N 186004454
Flow rate: 1 mL/min
Injection volume: 10 µL
Run Tim: 10-min
System suitability solution: 4 µg/mL each of USP Cetirizine Hydrochloride RS and USP Cetirizine Related Compound A RS in Mobile phase
Sys Suit Requirements: Tailing is NMT 2.0
Resolution between Cetirizine HCl and Cetirizine RC A is NLT 2.0
System 1: Arc™ HPLC system with 2489 UV Detector and CHC
System 2: Vendor X HPLC

Needle Wash:
a: Acetonitrile:Water (93:7)
b: Acetonitrile:Water (80:20)
c: Acetonitrile:Water (60:40)
d: Acetonitrile:Water (40:60)
e: Acetonitrile:Water (20:80)
f: Acetonitrile:Water (7:93)

Note: Needle wash line was flushed for 6 cycles before each analysis

RESULTS AND DISCUSION

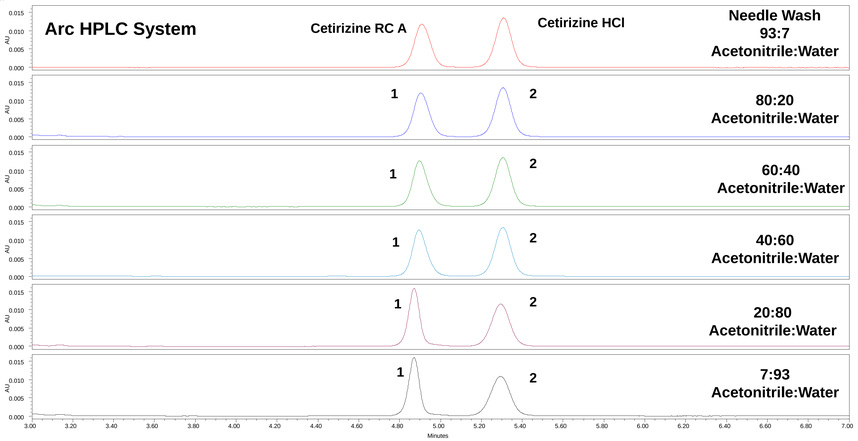

Fig 1: Impact of Needle Wash on HILIC USP Monograph on Arc HPLC System. **Peak 1**: Cetirizine RC A **Peak 2**: Cetirizine HCl

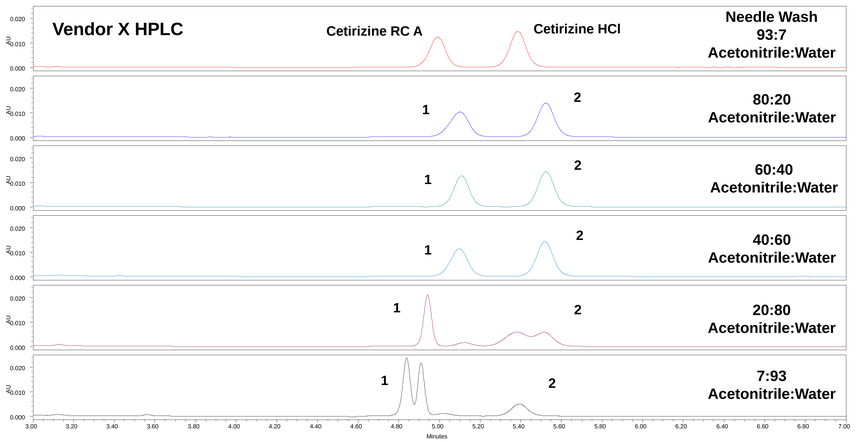

Fig 2: Impact of Needle Wash on HILIC USP Monograph on Vendor X HPLC. **Peak 1**: Cetirizine RC A **Peak 2**: Cetirizine HCl

For this study, 6 different needle wash solutions were prepared to be used for analysis. Two HPLC systems, Arc HPLC System and Vendor X HPLC systems, where 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.

The System Suitability Solution was analyzed on the two HPLC systems. Each system was equilibrated for 24-hours before analysis. The same column, mobile phases, and needle wash solutions were used on both systems. 3 replicate injections of the sample was acquired for each needle wash composition. Before each 3 replicate injections, the needle wash was flushed for 6 cycles to ensure the prior needle wash was fully replaced by the new wash.

A

Arc HPLC System: 93:7 (Acetonitrile:Water)	Tailing NMT 2.0	Resolution NLT 2.0
Cetirizine RC A	1.0	
Cetirizine HCl	1.0	2.7

B

Arc HPLC System: 7:93 (Acetonitrile:Water)	Tailing NMT 2.0	Resolution NLT 2.0
Cetirizine RC A	1.0	
Cetirizine HCl	1.0	3.0

C

Vendor X HPLC System: 93:7 (Acetonitrile:Water)	Tailing NMT 2.0	Resolution NLT 2.0
Cetirizine RC A	1.0	
Cetirizine HCl	1.0	2.6

Table 1: Sys Suit Requirement results on Arc HPLC System (**A** and **B**) and Vendor X HPLC (**C**) for various needle wash compositions examined. Results are an average of 3 replicate injections

Figure 1 displays the System Suitability chromatograms obtained using the Arc 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 1A and 1B for 93:7 Acetonitrile:Water and 7:93 Acetonitrile:Water respectively.

Figure 2 displays the System Suitability chromatograms obtained using the Vendor X HPLC for all needle wash compositions examine. 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. Table 1C shows that Vendor X's HPLC system meets system suitability requirements with the less aqueous wash composition of 7:93 Acetonitrile:Water, but due to peak splitting seen for the 20:80 and 7:93 Acetonitrile:Water, system suitability couldn't be calculated.

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.

CONCLUSIONS

- Although needle wash considerations are made to reduce carryover, this study provides an example where carryover is not the only critical parameter impacted by the needle wash.
- The needle wash can also impact the peak shape or chromatography. Due to the mechanical designs, specifically the autosampler of the Vendor X HPLC system, the peak splitting creates a failing system suitability.
- The Arc HPLC System autosampler design doesn't exhibit any interference from the needle wash with the sample allowing system suitability to be met regardless of aqueous content in needle wash.
- Migration of the Cetirizine Hydrochloride USP Organic Impurities Monograph from Arc HPLC System to Vendor X HPLC system is unsuccessful due to a highly aqueous needle wash composition creating chromatographic problems.

REFERENCES

1. Cetirizine Hydrochloride USPNF 2021 ISSUE 1 – online, USP43-NF38 – 910, USP42-NF37 - 891

TO DOWNLOAD A COPY OF THIS POSTER, VISIT WWW.WATERS.COM/POSTERS