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Application Note

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration Part 2: Troubleshooting Peak Splitting of Cetirizine

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Abstract

Lack of familiarity with hydrophilic interaction liquid chromatography (HILIC) methods, which are known for their unique and complex separation mechanisms, can pose challenges for system set ups given the differences in strong and weak solvents as compared to reversed-phase LC. This challenge may be more prevalent when setting up a pharmacopeial analysis since only key method parameters are included in the monograph. A method parameter such as needle wash composition is not typically included and thus should be evaluated and optimized by the end user. Additionally, due to differences in HPLC instrument design, a system that does not prevent interaction of washes with the flow path has the potential to impact chromatography. A well-designed system like the Alliance™ iS HPLC System enables the use of a very strong HILIC wash solvent by ensuring there is no interaction between the sample flow path and the wash solvent. For this study, the system suitability solution sample from the cetirizine hydrochloride organic impurities USP monograph was used to assess the impact of the needle wash and needle wash design for the Alliance iS HPLC System and a comparable HPLC system (referred to as Vendor X HPLC) on HILIC separations.

Benefits

- Improved needle washing mechanism on the Alliance iS HPLC System
- HILIC Method Migrations

Introduction

Most regulated laboratories have numerous HPLC systems, which may have different designs and characteristics. Because of these design differences, moving methods across systems requires testing to ensure comparable results are generated and the workflow is not impacted. One type of method executed in these regulated laboratories are HILIC methods which is used for separating polar compounds. 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 cetirizine hydrochloride organic impurities USP method, system suitability could not be met due to a split peak being observed in the system suitability solution sample on one of the systems.[1,2] Because the presence of excess water in HILIC methods 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 Alliance iS HPLC System and Vendor X HPLC System can affect the results of a HILIC method analysis when varying wash solvent compositions are utilized.

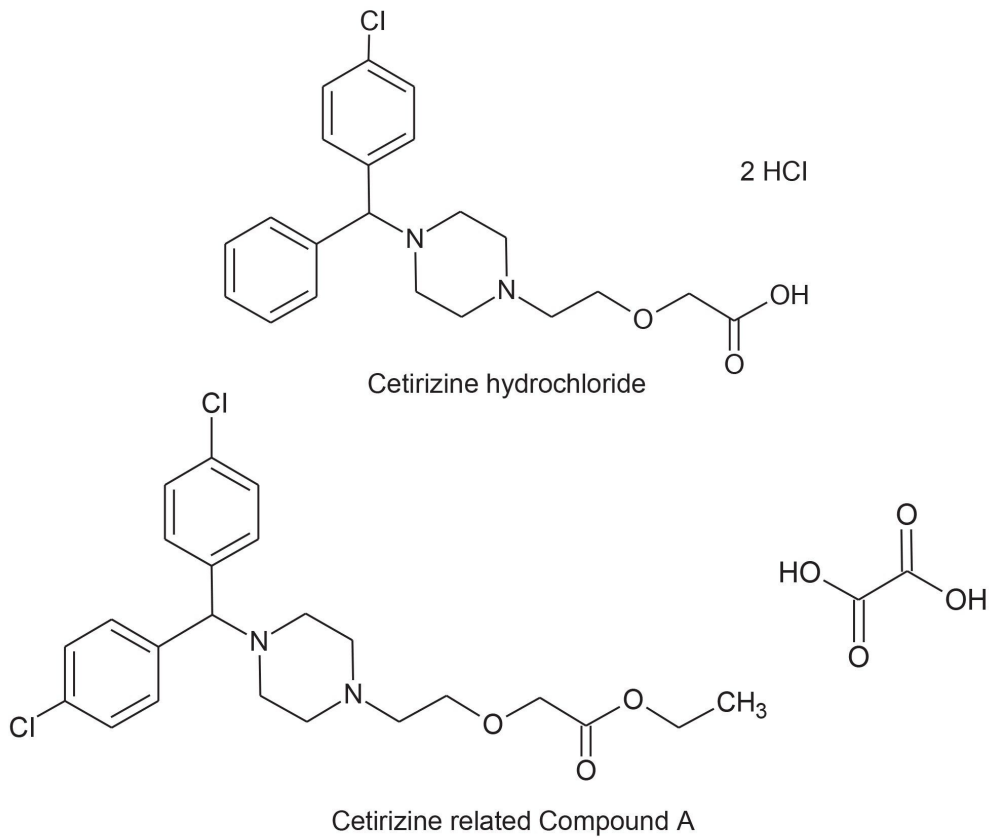

Figure 1. Structures of Cetirizine HCl and Cetirizine RC A.

Experimental

Sample Description

Two reference standards were obtained from Sigma-Aldrich: Cetirizine Hydrochloride (CAS No.: 83881-52-1) and Cetirizine Related Compound A (RC A) (CAS No.: 246870-46-2). System Suitability Solution consists of 4.0 µg/mL Cetirizine Hydrochloride and 4.0 µg/mL Cetirizine RC A in mobile phase.

LC Conditions

LC system:	1. Alliance™ iS HPLC System 2. Vendor X HPLC System
Detection:	1. Dual Wavelength UV Detector, 230 nm at 10 points/second 2. Variable Wavelength Detector (VWD), 230 nm at 10 *Hz*
Vials:	TruView™ pH Control LCMS Certified Max Recovery Vials, p/n: 186005662CV
Column(s):	XBridge™ BEH™ HILIC Column, 130 Å, 5-µm, 4.6 × 250 mm (p/n: 186004454)
Column temperature:	25 °C
Sample temperature:	10 °C
Injection volume:	10 µL
Flow rate:	1.0 mL/min
Mobile phase:	Acetonitrile (ACN): water: and 1 M sulfuric acid (93:6.6:0.4)
Needle wash:	A. ACN: water (93:7) B. ACN: water (80:20) C. ACN: water (60:40) D. ACN: water (40:60) E. ACN: water (20:80)

	F. ACN: water (7:93)
Method:	*Organic Impurities: Isocratic 18-minute method*

Data Management

Chromatography software:	Empower™ 3 FR4 Empower 3.7

Results and Discussion

Troubleshooting of Peak Splitting in HILIC Separation

While migrating the USP Cetirizine Hydrochloride Organic Impurities method to Vendor X HPLC System, peak splitting was observed with the system suitability solution. Initial troubleshooting focused on the column and ensuring proper equilibration of the column and system prior to analysis, given the long equilibration times required for HILIC separations. Other L3 columns were tested for analysis but yielded similar peak splitting issues. Other common sources of error were considered including sample preparation and mobile phase preparation to identify any potential solvent mismatch effects or a mismatch between the sample diluent and the method starting conditions. Per the USP method, the system suitability sample is prepared in mobile phase, therefore there should be no chance of solvent mismatch. Analysis of the freshly prepared samples and mobile phases showed similar peak splitting, indicating that neither were the root cause of the peak splitting.

Since the method being migrated was a HILIC method, where water is the strong solvent, the presence of excess water could negatively impact the chromatography due to strong solvent effects.[3] Because the autosampler was being controlled at 10 °C, it was theorized that condensation may be forming and introducing water into the sample vial over time. A freshly prepared sample, along with a previously prepared sample that had been stored in the autosampler at 10 °C were analyzed. For this analysis, the autosampler temperature control was turned off to prohibit any condensation from forming. The resulting chromatography for both samples showed the now

familiar peak splitting issues, confirming that condensation was also not the root cause of the chromatographic problem.

The final troubleshooting step that was examined was to remove the needle washing step. The needle wash used for this method migration contained a high percentage of water but had not shown any problematic behavior on the LC systems previously used. Interestingly, when the needle wash step was removed, the peak splitting issue resolved. To confirm the use of needle washing as the source of the peak splitting, turning the needle washing back on resulted in the return of the peak splitting.

Based on the observed impact of the wash solvent, a study was then developed to explore how the design of the needle wash mechanism and needle wash composition could affect chromatography.

Study Design

It is common for needle wash compositions to be made relatively strong to mitigate carryover. Because this method is a HILIC method, the higher the aqueous content, the higher the solvent strength will be. For this study, 6 needle wash solutions with varied water to acetonitrile ratio were evaluated and ranged from 93:7 water:acetonitrile (strongest) to 93:7 acetonitrile:water (weakest). Two HPLC systems with significantly different needle washing mechanisms were used to illustrate the impact needle wash design and mechanism may have on chromatography. The two systems evaluated in this study include an Alliance iS HPLC System and Vendor X HPLC System.

The Cetirizine Hydrochloride Organic Impurities USP method was the HILIC method used to assess the impact of the needle washes. Because meeting system suitability requirements is a key factor when running any compendial method, the system suitability solution was used to evaluate which HPLC system and needle wash compositions could be successfully migrated.

Before analysis, each system was equilibrated at initial conditions for 24 hours using the same column and mobile phases. For each needle wash composition evaluated, the needle wash solvent was primed for sufficient time to ensure complete solvent changeover in the lines and system. Three replicate injections of the system suitability sample were acquired following a blank injection. After the third replicate sample injection, the needle wash solution was changed to a new composition, and again primed for sufficient time before the next analysis began.

Once analysis using all 6 needle wash compositions was completed, all chromatograms were reviewed and processed. Per the monograph, the system suitability solution should have a tailing of not more than (NMT) 2.0

and the resolution between Cetirizine Hydrochloride and Cetirizine Related Compound A is not less than (NLT) 2.0

Impact of Needle Wash Design on HILIC Separations

The stacked chromatograms in Figure 2 display a single injection of the system suitability sample obtained on the Alliance iS HPLC System using the six needle wash compositions.

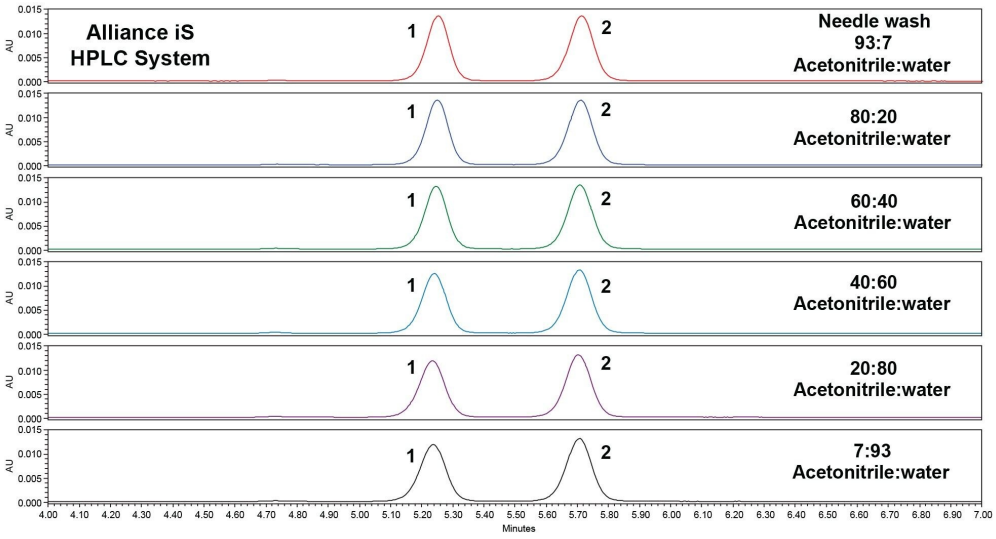

Figure 2. Cetirizine HCl system suitability solution chromatograms obtained using various needle wash compositions on the Alliance iS HPLC System. Peak 1: Cetirizine RC A. Peak 2: Cetirizine HCl.

All the chromatograms show symmetrical peak shape and good resolution between the cetirizine related compound A and cetirizine peaks. The average peak tailing and resolution for the three injections at each wash composition are reported in Tables 1 and 2. Regardless of the strength of the needle wash composition, with a higher amount of aqueous being stronger, all system suitability requirements were met as per the monograph.

Table 1

Alliance iS HPLC System		
Needle wash	System suitability solution	
	Tailing factor (NMT 2.0)	Resolution (NLT 2.0)
ACN : water (93:7)	1.0	3.2
ACN : water (80:20)	1.0	3.2
ACN : water (60:40)	1.0	3.2
ACN : water (40:60)	1.0	3.1
ACN : water (20:80)	1.0	3.0
ACN : water (7:93)	1.0	2.9

The same methodology was run on the Vendor X HPLC System and example system suitability chromatograms are displayed in Figure 3. It is readily apparent that for the last two needle wash compositions evaluated, there is significant peak splitting seen.

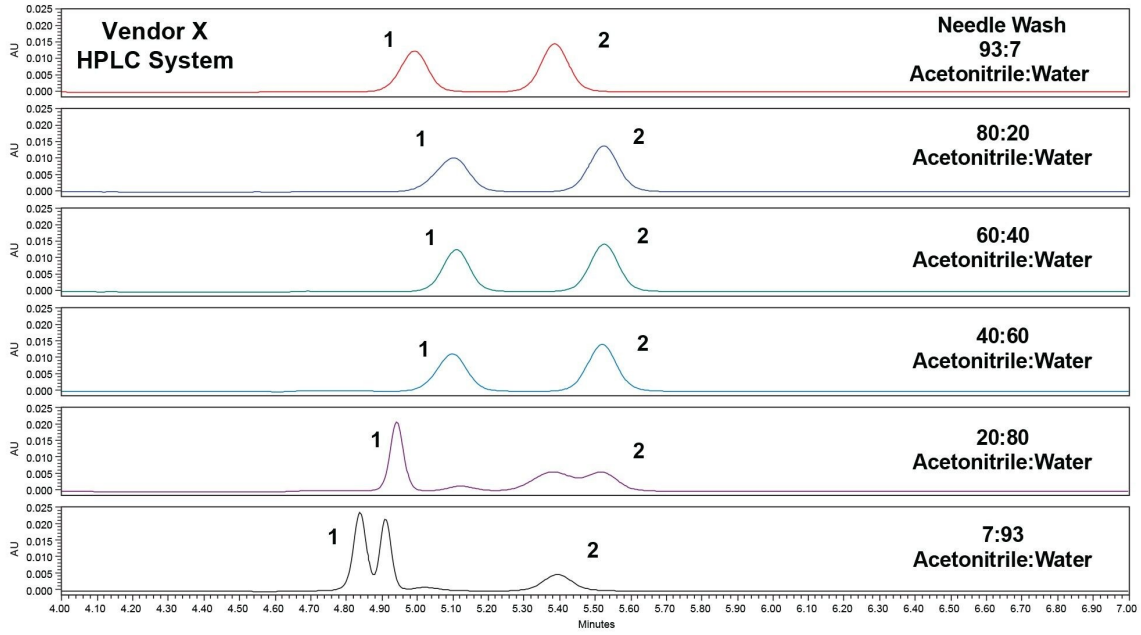

Figure 3. Cetirizine HCl system suitability sample chromatograms obtained using various needle wash compositions on the Vendor X HPLC System.
Peak 1: Cetirizine RC A. Peak 2: Cetirizine HCl.

Needle wash compositions of 93:7, 80:20, 60:40, and 40:60 Acetonitrile:Water illustrate acceptable chromatograms while also meeting system suitability requirements as observed in Table 2. Compositions of 20:80 and 7:93 Acetonitrile Water produced unacceptable chromatograms due to significant peak splitting, thus not meeting the system suitability requirements (Table2).

Table 2

Vendor X HPLC System		
Needle wash	System suitability solution	
	Tailing factor (NMT 2.0)	Resolution (NLT 2.0)
ACN : water (93:7)	1.0	2.6
ACN : water (80:20)	1.0	2.3
ACN : water (60:40)	1.0	2.8
ACN : water (40:60)	1.0	2.5
ACN : water (20:80)	Could not Process	Could not Process
ACN : water (7:93)	Could not Process	Could not Process

Examination of the washing mechanism for both the Alliance iS HPLC System and Vendor X HPLC System provides insight into the peak splitting observed on the Vendor X HPLC System. For the Vendor X HPLC, the needle wash sequence involves the needle moving from the vial after the required amount of sample has been drawn and then moving into a wash port where an amount of needle wash is present. The exterior of the needle is then washed, and the needle is moved to the needle seat to begin injection. Due to the way the Vendor X HPLC System washes the needle, an amount of the wash is being injected along with the sample into the system since the needle moves directly from the wash station and into the inject port. Because HILIC methods are very sensitive to the presence of water, the strong aqueous washes present the chromatographic issues observed due to the wash mechanism of the Vendor X HPLC System.

On the Alliance iS HPLC System, the design ensures that the needle is properly washed, however the needle wash is not introduced into the flow path with the sample. Specifically, the needle arm moves from the sample vial and into the injection station where the needle is washed, and sample is injected. The wash solvent is delivered by two lines at both the top and the bottom of the wash station. At the start of the wash cycle, the puncture needle, along with the sample needle, enters the station through the top and remains near the top wash solvent port. The wash is then introduced through both top and bottom port while washing the puncture needle, sample needle, and the space between them. The wash is aspirated out and the sample needle moves down into the seal at high pressure to begin injection while the puncture needle remains near the top wash port. During injection, more wash is introduced and aspirated out while washing the remaining parts of the sample needle. The wash continues after sample injection is completed and the needle moves back to the top of the wash station. The result is no observable peak splitting on the Alliance iS HPLC System, regardless of needle wash composition.

Conclusion

Migrating a HILIC method such as the Cetirizine Hydrochloride USP Organic Impurities monograph across systems using a strong aqueous wash can be unsuccessful potentially due to the difference in wash mechanism of the systems. The Alliance iS HPLC System is designed to help minimize carryover as well as limit the introduction of the needle wash into the sample matrix. Regardless of the wash solvent being used, the Alliance iS HPLC System can properly wash the needle while mitigating the introduction of the wash solvent into the injection and providing acceptable chromatograms to be analyzed.

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 < https://doi.usp.org/USPNF/USPNF_M2902_06_01.html> .
2. Pedanou E, Hong P, Lander N. Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration Part 1: From Legacy HPLC Systems to the Alliance™ iS HPLC System. Waters Application Note. 720007993. June 2023.
3. Malm M, Kjellström J. Elimination of the Sample Solvent Effect when Analysing Water Solutions of Basic Peptides by HILIC. *Chromatography Today.* February / March 2020, Pages 28–30.

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