Waters™

Application Note

Hydrophilic Interaction Liquid Chromatography (HILIC) Method Migration Part 1: From Legacy HPLC Systems to the Alliance™ iS HPLC System

Elom Y Pedanou, Paula Hong, Neil J. Lander

Waters Corporation

Abstract

This application note helps illustrate a successful hydrophilic interaction liquid chromatography (HILIC) method migration from legacy HPLC systems found across industries to the Alliance iS HPLC System. Due to differences in HPLC instrument designs, HILIC method migration could be challenging since results could

potentially be impacted. A well-designed system like Alliance iS HPLC System, helps overcome those challenges to allow for seamless migration. For this study, the United States Pharmacopeia (USP) monograph for cetirizine hydrochloride assay and organic impurities were analyzed on two legacy HPLC systems and migrated to the Alliance iS HPLC System. All three systems met suitability requirements and demonstrated comparable results.

Benefits

- Seamless HILIC method migration from standard HPLC systems to Alliance iS HPLC System
- Improved signal-to-noise (s/n)

Introduction

Most regulated laboratories, such as pharmaceutical quality control (QC) labs, utilize high performance liquid chromatography (HPLC) systems for analysis of intermediate and final products. When considering a new HPLC system, the ability to migrate existing validated methods is critical. Specific attributes of the method may add complexity to this process. For example, hydrophilic interaction liquid chromatography (HILIC) separations use high organic mobile phases, and different washes than used for the more typical reversed-phase chromatography (RP). This results in specific considerations and challenges associated with HILIC methods.

In this study, the Cetirizine Hydrochloride assay and organic impurities monograph, from the USP, is used to

assess HILIC method migration from legacy HPLC systems to the Alliance iS HPLC System.[1] The performance of the method will be evaluated by analysis of the system suitability requirements: Tailing factor, resolution, signal-to-noise (s/n), peak area %RSD, and retention time %RSD.

Experimental

Assay

The standard solution was prepared at 0.5 mg/mL Cetirizine Hydrochloride (CAS No.: 83881-52-1) in mobile phase, as described in the USP monograph.

Organic Impurities

- The System Suitability Solution was prepared at 4.0 µg/mL Cetirizine Hydrochloride and 4.0 µg/mL Cetirizine Related Compound A (CAS No.: 246870-46-2) in mobile phase
- The Sensitivity Solution was prepared at 0.1 µg/mL Cetirizine Hydrochloride in mobile phase
- The Standard Solution was prepared at 0.5 µg/mL Cetirizine Hydrochloride in mobile phase

LC Conditions

LC system:	1. Legacy System 1 2. Legacy System 2 3. Alliance iS HPLC System

Detection:	1. 2489 UV/Vis Detector, 230 nm @ 10 points/second 2. Variable Wavelength Detector (VWD), 230 @ 6.87 Hz 3. Dual Wavelength UV Detector, 230 nm @ 10 points/second
Vials:	TruView pH Control LCMS Certified Max Recovery Vials, (p/n: 186005662CV)
Column(s):	XBridge™ BEH™ HILIC Column, 130 Å, 5-µm, 4.6 mm × 250 mm (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, and 1 M sulfuric acid (93:6.6:0.4)

Needle wash:	ACN, water (93:7)
Method:	*Assay: Isocratic 10-minute method* *Organic Impurities: Isocratic 18-minute method*

Data Management

Chromatography software:	Empower™ 3 FR4 Empower 3.7

Results and Discussion

For the Cetirizine Hydrochloride Monograph, both the assay and organic impurities methods require the same mobile phase and column, enabling sequential analysis. In this study, the mobile phase and samples were prepared prior to each analysis, and a new column was used for each system. Each new column was conditioned for 12 hours prior to analysis. All HPLC systems followed a similar sample sequence. The mobile phase and the assay standard were used for the assay analysis while the system suitability solution, sensitivity solution, organic impurities standard solution, and mobile phase were used for the organic impurities analysis.

The assay results, which include injection of the standard, demonstrated the ability to successfully move the method across HPLC systems. The system suitability requirements (Table 1) associated with the assay standard include relative standard deviation (RSD) for peak area and retention time of Not More Than (NMT) 0.73% and tailing factor of NMT 2.0 (Table 1). For this isocratic method, retention time and tailing factor may be impacted by the column and method conditions, as HILIC methods typically require long equilibration times. Alternatively, the area %RSD is most likely to be impacted by the injection precision of the system and can be challenging to meet with the combination of the high organic sample diluent (>93% acetonitrile) and a low Area %RSD requirement (NMT 0.73%). While all three systems met the system suitability criteria, the Alliance iS HPLC System, with newly designed sample metering pump, produced the lowest overall area RSD of 0.042%.

The organic impurities method included a standard, a system suitability solution and a sensitivity solution, the latter to ensure the system is suitable to quantify low levels of impurities. The suitability requirements were retention time and peak area of NMT 2.0% (standard), signal-to-noise of Not Less Than (NLT) 10 (sensitivity solution), USP tailing ≤2, and USP resolution ≥2 for cetirizine and cetirizine related compound A (system suitability solution). As shown (Figure 1) all systems produced comparable chromatography. While all systems met the suitability requirements (Table 2), the s/n on the Alliance iS HPLC System was greater than the legacy systems while also achieving the lowest peak area RSD.

Cetirizine hydrochloride assay				
Criteria	USP specification	HPLC system		
		Legacy system 1	Legacy system 2	Alliance iS HPLC System
Area %RSD	NMT 0.73%	0.220	0.082	0.037
Retention time %RSD	NMT 0.73%	0.018	0.021	0.327
USP tailing	NMT 2.0	0.737	0.688	0.649

Table 1. System Suitability Results for Cetirizine Hydrochloride Assay on Legacy Systems and Alliance iS HPLC System.

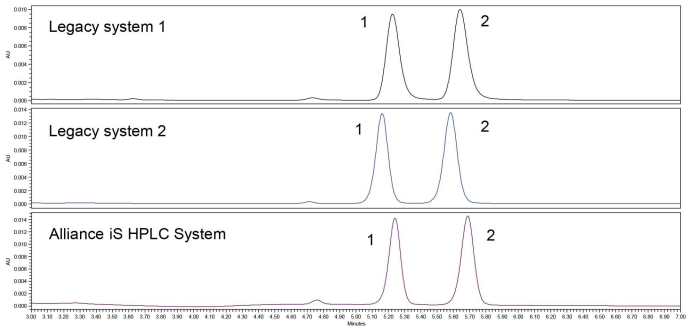

Figure 1. Representative chromatograms of the Organic Impurities System Suitability Solution on all three HPLC systems.
Peak 1 = Cetirizine Related Compounds A. Peak 2 = Cetirizine Hydrochloride

Cetirizine hydrochloride organic impurities				
Criteria	USP specification	HPLC system		
		Legacy system 1	Legacy system 2	Alliance iS HPLC System
Area %RSD	NMT 2.0%	0.501	0.748	0.215
Retention time %RSD	NMT 2.0%	0.030	0.017	0.015
System suitability solution				
USP tailing	NMT 2.0	1.205	0.981	1.127
USP resolution	NLT 2.0	2.569	2.842	2.649
Sensitivity solution				
USP signal-to-noise	NLT 10	30.53	21.32 (85.53)	96.84

Table 2. System Suitability Results for Cetirizine Hydrochloride Organic Impurities on Legacy Systems and Alliance iS HPLC System.

Note: Legacy System 2 USP signal-to-noise in parentheses () was calculated using noise centered around specified region of blank (See section below).

Calculating Signal-to-noise Using Custom Calculations: Impact of Carryover

With recent changes to the USP General Chapter <621>, current guidelines specify signal-to-noise (s/n) calculated using a blank injection, with the noise region centered–if possible-on the peak retention time using a half height multiplier of 5.[2] However, with the need of a blank, any extraneous peaks in the blank can impact s/n calculations, including solvent peaks or carryover.

In this analysis, s/n was calculated from the blank at 5x the peak width at half height, centered around the peak. While all systems met system suitability criteria, Legacy System 2 produced the lowest sensitivity. Upon review of the data, a peak was observed in the blank chromatogram of Legacy

System 2, near the elution of the analyte of interest (Figure 2). The retention time alignment with the peak of interest, suggest it is likely due to carryover. While modern HPLC methods have various needle washing designs, many legacy HPLC systems have few options to wash any previous samples from the injection needle. In contrast, during an injection cycle of the Alliance iS HPLC System, the sample needle and puncture needle are washed before and after injections with the wash solvent, continually being aspirated out. Thus, the Alliance iS HPLC System showed no observable peaks in the blank, providing improved s/n (Figure 3).

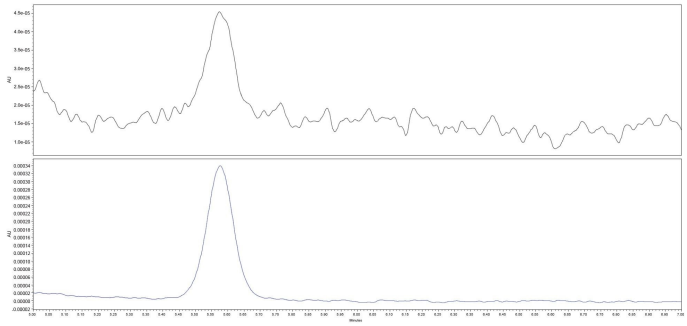

Figure 2. Representative chromatograms for blank containing carryover (top) and Sensitivity Solution (bottom) used to calculate signal-to-noise on Legacy system 2.

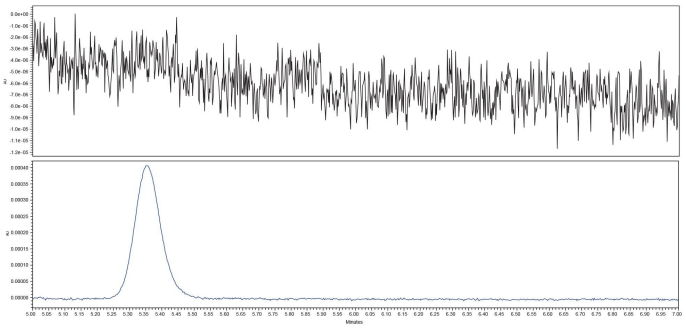

Figure 3. Representative chromatograms for blank (top) and Sensitivity Solution (bottom) used to calculate signal-to-noise on the Alliance iS HPLC System.

While Legacy System 2 met system suitability criteria, an alternative methodology to calculate s/n results without any carryover interference was evaluated. This methodology used a different region of the blank not affected by carryover. To perform the calculation, a custom field was used. The custom field was created as follows:

1. Label "Blank" in the Sample Set Method (Figure 4).

Inj Vol (uL)	# of Injs	Label	SampleName	Sample Type	Level	Function	Method Set / Report or Export Method
10.0	1		Blank	Unknown		Inject Samples	Cetirizine OI MS
10.0	1	Blank	Blank	Unknown		Inject Samples	Cetirizine OI MS
10.0	6		Sensitivity Solution	Unknown		Inject Samples	Cetirizine OI MS
10.0	2		Blank	Unknown		Inject Samples	Cetirizine OI MS

Figure 4. Single Blank injection to be used or s/n calculation with Label definition.

2. Create the custom field using intersample syntax with the label definition for the *blank injection* in the formula field box (Figure 5). The full formula is as follows:

2*(*Height*-(0.5*Blank.1.(*Peak to Peak Noise*/*Scale to µV*
)))/Blank.1.(*Peak to Peak Noise*/*Scale to µV*).

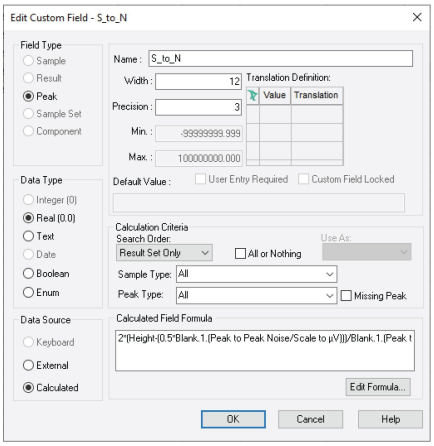

Figure 5. Custom Field for s/n calculation using the blank.

3. Set the Noise Value for s/n specified in suitability tab of the processing method. For this custom field, the Noise value for s/n must be Peak to Peak Noise (Figure 6).

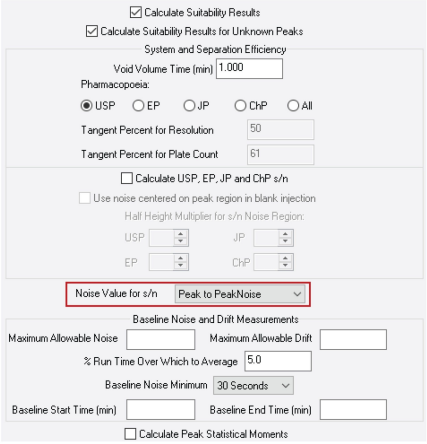

Figure 6. Suitability Tab of the processing method.

4. Next set the appropriate segment to use for the blank in the Noise and Drift tab of the processing method (Figure 7).

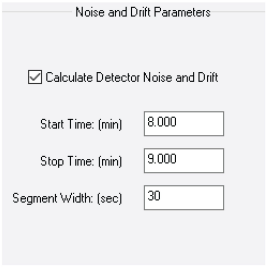

Figure 7. Noise and Drift tab of the processing method with chosen segment on the blank.

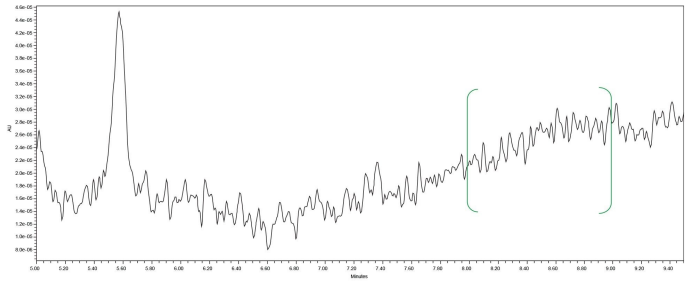

Figure 8. Segment in Blank injection selected for s/n calculation.

Using this intrasample custom calculation, the s/n results increased from 21.32 to 85.53 for Legacy System 2 after re-processing, demonstrating the impact of extraneous peaks in a blank s/n value.

Conclusion

Modern HPLC systems such as the Alliance iS HPLC System offer many benefits such as successfully running methods from legacy HPLC systems and producing similar and even improved results as observed with s/n. A HILIC method, which can be present issues in routine analysis, was able to be successfully migrated from legacy HPLC systems to the Alliance iS HPLC System. All suitability criteria were met for migration of the Cetirizine Hydrochloride Assay and Organic Impurities USP method with the Alliance is HPLC System having comparable chromatography as Legacy systems with slightly improved area %RSD's and sensitivity. In addition, due to the improved needle wash mechanism on the Alliance iS HPLC

System, no carryover was observed.

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. General Chapter: USP. 621 Chromatography. In: USP-NF. Rockville, MD: USP; 01 Apr 2023. DOI: https://doi.usp.org/USPNF/USPNF_M99380_07_01.html < https://doi.usp.org/USPNF/USPNF_M99380_07_01.html >

3. "How to Create an Intersample Custom Field - Tip93." *Waters*, 11 Nov. 2022, https://support.waters.com/KB_Inf/Empower_Tips_of_the_Week/WKB68884_How_to_create_an_Intersample < https://support.waters.com/KB_Inf/Empower_Tips_of_the_Week/WKB68884_How_to_create_an_Intersample >

Featured Products

Alliance iS HPLC System < https://www.waters.com/534293>

ACQUITY UPLC Tunable UV Detector <

https://www.waters.com/514228>

2489 UV/Visible (UV/Vis) Detector <

https://www.waters.com/515198>

Empower Chromatography Data System <

https://www.waters.com/10190669>

720007993, June 2023