

Scaling and Migration of a Hydrophilic Interaction Liquid Chromatography (HILIC) Method for Related Compounds of Ribavirin to Modern HPLC Systems

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Abstract

Achieving identical chromatographic separation and equivalent results are critical for a successful method scaling across different particle size columns and migration between LC systems. In this work, a HILIC method for the analysis of active pharmaceutical ingredient (API), ribavirin, and related compounds was scaled from a 1.7 μm (2.1 x 50 mm) column to 2.5 μm (3.0 x 75 mm), and 3.5 μm (4.6 x 100 mm) columns with equivalent chemistry. The method was then migrated from an ACQUITY™ UPLC™ H-Class PLUS System to ACQUITY Arc™, Arc HPLC, and Alliance™ iS HPLC systems. Equivalent performance was demonstrated by assessing chromatographic separation, system suitability, and assay results generated by the method on the different systems.

Benefits

- Methods can be successfully migrated between Waters™ UHPLC and HPLC systems
- The Waters Columns Calculator assists in method scaling by calculating operating conditions to generate identical separation and performance

- The Gradient SmartStart feature in Empower compensates for the differences in system dwell volume, eliminating the need for manual adjustments to the gradient table when migrating methods

Introduction

Analytical methods are often developed on sub-2 μm particle size columns to increase the speed of the method development process and reduce analytical run time. These methods generally require a low-dispersion liquid chromatography (LC) system to achieve optimum chromatographic separation. Often methods are transferred to quality control (QC) laboratories that are not equipped with low dispersion systems. In this case, there is a need to scale methods to larger particles designed for use with higher-dispersion HPLCs.¹ The receiving laboratory needs to demonstrate equivalent results for the same analysis to ensure product consistency and compliance with the regulatory guidelines.²

Scaling and migration of chromatographic methods between different LC systems can be a challenging task. Often, systems are characterized by different dwell and extra-column volumes, which may result in poor chromatographic separation and peak distortion in gradient methods, subsequently producing inconsistent results for the scaled/migrated assay. The differences in system characteristics must be considered when scaling and migrating methods.³

This work demonstrates the scaling and migration, to modern HPLC systems, of a HILIC method for the analysis of ribavirin and related compounds.⁴ Ribavirin is an antiviral drug used to treat chronic hepatitis C virus (HCV) infection.⁵ The method was previously developed on a sub-2 μm particle sized column. In this work, the method is scaled from 1.7 to 2.5 and 3.5 μm particle columns. The method is then migrated from an ACQUITY UPLC H-Class PLUS System (1.7 μm column) to ACQUITY Arc (2.5 μm), Arc HPLC and Alliance iS HPLC systems (3.5 μm). Performance characteristics including chromatographic separation, system suitability, and assay results are examined on each system to measure the success of method scaling and migration.

Experimental

Sample Description

Standard Solutions

Neat standards of ribavirin and related compounds (Figure 1.) were purchased from Sigma-Aldrich. Individual stock solutions were prepared in 50:50 acetonitrile/water at 1.0 mg/mL. Stock solutions were diluted with 90:10 acetonitrile/water diluent to make a mixture standard solution with ribavirin API at 0.1 mg/mL and related compounds at 10 µg/mL.

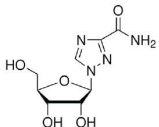
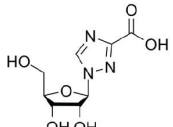
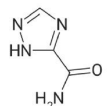
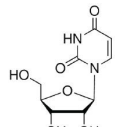
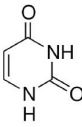
Ribavirin	Ribavirin related compound A (RGA)	Ribavirin related compound D (RGD)	Uridine	Uracil
$C_8H_{12}N_4O_5$ MW: 244.20	$C_8H_{11}N_3O_6$ MW: 245.19	$C_3H_4N_4O$ MW: 112.09	$C_9H_{12}N_2O_6$ MW: 244.20	$C_4H_4N_2O_2$ MW: 112.09
				

Figure 1. Chemical information of ribavirin API and related compounds. MW: molecular weight.

Method Conditions

Mobile phase A:	Acetonitrile
Mobile phase B:	Water
Mobile phase C:	100 mM ammonium bicarbonate with 1% ammonium hydroxide solution (pH 10)
Column temperature:	30 °C
Detection:	UV at 220 nm
Vials:	LCMS Maximum Recovery 2 mL volume, p/n: 600000670CV

Sample temperature:

10 °C

Wash solvents:

Purge/sample/needle: 90:10 acetonitrile/water

Seal wash: 10:90 acetonitrile/water

Method conditions used for 1.7 and 2.5 and 3.5 μm particle columns are summarized in Table 1. The flow rate, injection volume, and gradient elution were geometrically scaled using the Waters Columns Calculator.⁶

Parameter	Conditions for 1.7 μm column						Scaled conditions for 2.5 μm column						Scaled conditions for 3.5 μm column					
Systems	ACQUITY™ UPLC™ H-Class Plus System, ACQUITY™ PDA, active pre-heating						ACQUITY™ Arc™ System, 2998 PDA, passive pre-heater						• Arc™ HPLC System, 2998 PDA, passive pre-heater • Alliance™ iS HPLC System with TUV Detector, passive pre-heating					
Column	ACQUITY™ UPLC™ BEH™ Amide, 2.1 x 50 mm, 1.7 μm (P/N: 186004800)						XBridge™ BEH™ Amide, 3.0 x 75 mm, 2.5 μm (P/N: 186006094)						XBridge™ BEH™ Amide, 4.6 x 100 mm, 3.5 μm (P/N: 186004868)					
L/dp	29,412						30,000						28,571					
Flow rate	0.5 mL/min						1.0 mL/min						2.0 mL/min					
Gradient	Step	Time	%A	%B	%C	Curve	Step	Time	%A	%B	%C	Curve	Step	Time	%A	%B	%C	Curve
	1	Initial	90.0	5.0	5.0	Initial	1	Initial	90.0	5.0	5.0	Initial	1	Initial	90.0	5.0	5.0	Initial
	2	1.50	90.0	5.0	5.0	6	2	2.30	90.0	5.0	5.0	6	2	3.60	90.0	5.0	5.0	6
	3	3.00	30.0	65.0	5.0	6	3	4.60	30.0	65.0	5.0	6	3	7.20	30.0	65.0	5.0	6
	4	4.00	30.0	65.0	5.0	6	4	6.10	30.0	65.0	5.0	6	4	9.60	30.0	65.0	5.0	6
	5	4.10	90.0	5.0	5.0	6	5	6.20	90.0	5.0	5.0	6	5	9.70	90.0	5.0	5.0	6
	6	7.00	90.0	5.0	5.0	6	6	9.50	90.0	5.0	5.0	6	6	14.00	90.0	5.0	5.0	6
Inj. volume	1.0 μL						3.1 μL						9.6 μL					

Table 1. Operating conditions for method scaling from 1.7 to 2.5, and 3.5 μm particle columns.

Data Management

Chromatography software:

Empower™ 3 Feature Release 5 Service Release 5 (FR5 SR5)

Empower 3.8.0 with Alliance iS HPLC System

Data acquisition and processing performed using Empower Software. Report templates built-in in the Empower project were used to create summary reports of processed data.

Results and Discussion

Considerations: Scaling and Migrating Methods

Scaling and migrating methods between different LC platforms can be impacted by numerous factors. When scaling across different particle sizes, the ratio of column length (L) to particle size (dp) must be the same as the original method to ensure chromatographic separation of the method is preserved. Parameters including flow rate, injection volume, and gradient times are scaled geometrically to generate equivalent chromatographic separation and performance.

Instrument characteristics, including dwell and extra-column volumes, can impact the quality of the chromatographic separation and should be accounted for when migrating methods across different systems.³ Methods operated on sub-2 μm particle columns are generally run on systems with small dwell volumes and low dispersion compared to method that utilize 2.5 to 5 μm particle columns. Operating sub-2 μm particle columns on a large dwell volume and high dispersion system may produce poor chromatographic separation and peak distortion in gradient methods. This may generate different results for the same assay. The procedure for measuring dwell volume of an LC system is described in a white paper.⁷

Case Study: Ribavirin and Related Compounds

In this work, a HILIC method for ribavirin and related compounds was scaled from 1.7 to 2.5 and 3.5 μm particle columns using Waters Columns Calculator.⁶ The original method utilized a 50 mm long, 1.7 μm particle column, resulting in $L/dp = 29,412$. The method was scaled to 2.5 and 3.5 μm particles 75- and 100-mm length columns, producing L/dp of 30,000, and 28,571 respectively. Operating conditions for methods run using 1.7, 2.5, and 3.5 μm particle columns are listed in Table 1.

The ACQUITY UPLC H-Class PLUS System has a smaller dwell volume (0.375 mL) compared to the ACQUITY Arc (1.19 mL), Arc HPLC (1.35 mL), and Alliance iS HPLC (1.5 mL) Systems. Therefore, the gradient start for the target methods was adjusted using Gradient StartSmart technology within Empower. An example of gradient start time adjustment for the Arc HPLC System is shown in Figure 2. The gradient was adjusted to start 2200 μL after the injection. The Gradient SmartStart feature compensated for the differences in systems dwell volume, eliminating the need for manual adjustments to the gradient table.

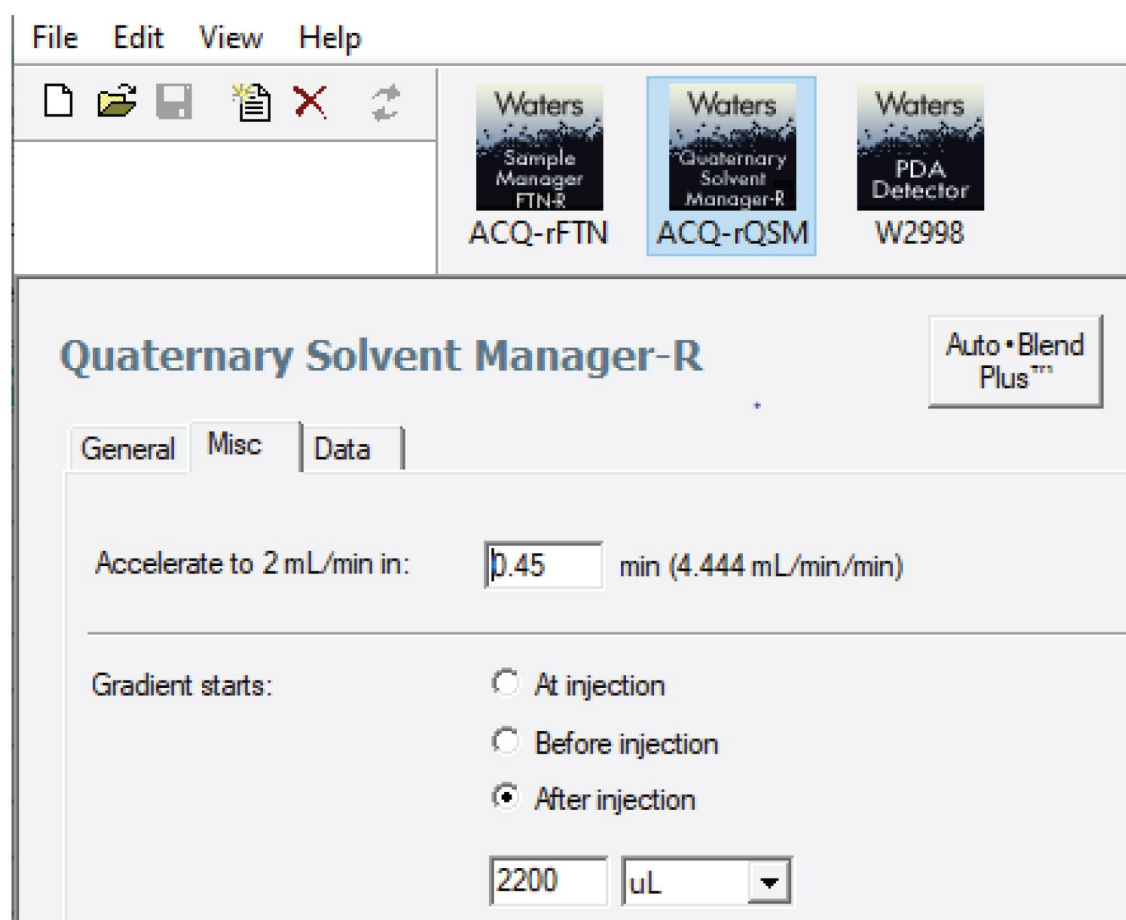


Figure 2. Example of Gradient StartSmart technology used to compensate for differences in system dwell volume during gradient methods migration. Empower instrument method for Arc HPLC system.

The chromatographic separation produced on the ACQUITY Arc, Arc HPLC, and Alliance iS HPLC systems was comparable to the data on the ACQUITY UPLC H-Class PLUS System (Figure 3). The first four peaks elute under isocratic conditions, while the last peak elutes (related compound A) during the gradient. The separation parameters including the retention times, retentivity factor (k^*), and USP Resolution (R_s) between peaks were comparable across all four systems (Table 2).

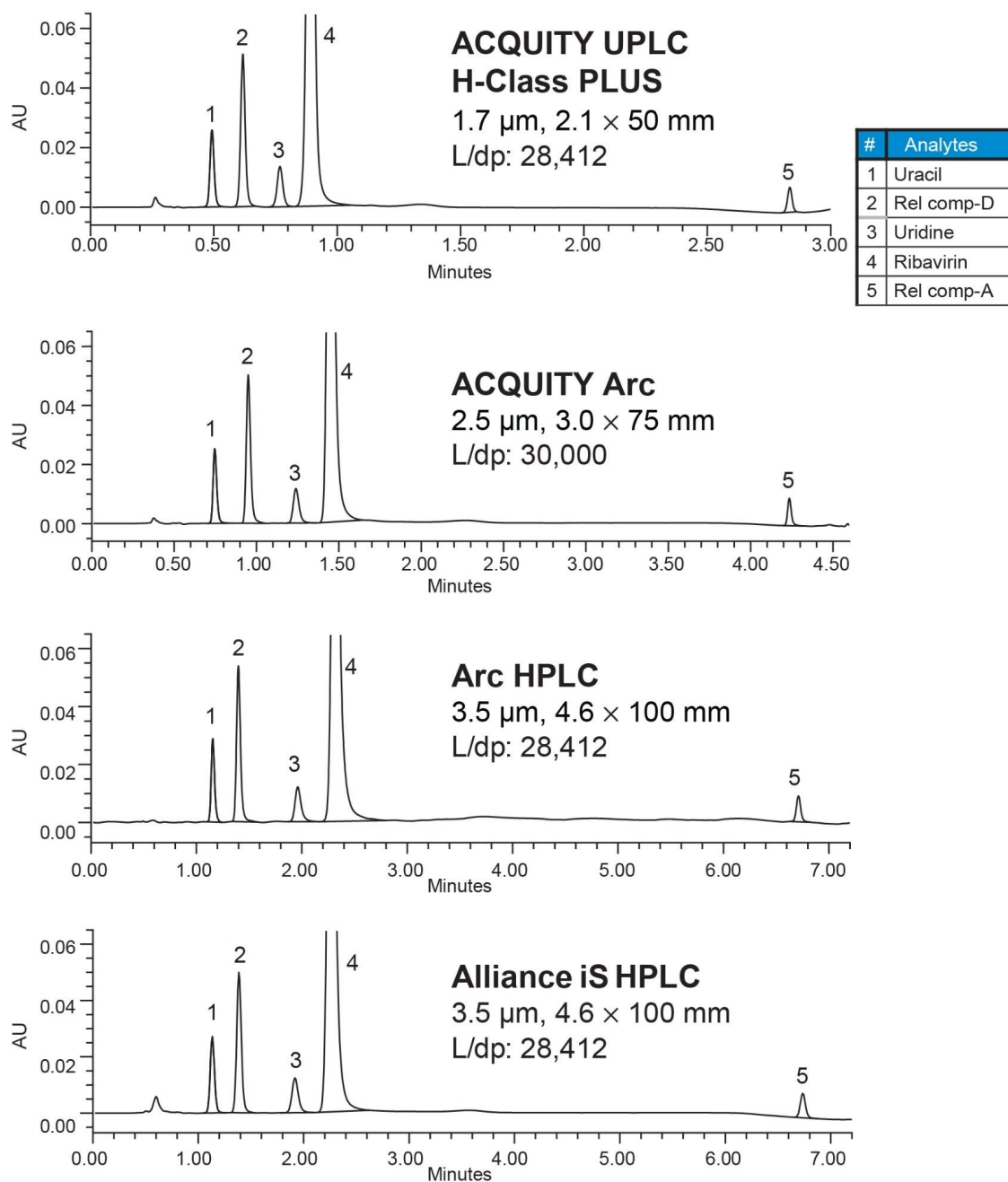


Figure 3. Representative chromatographs for method scaling and migration between different systems. Standard solution with ribavirin API at 0.1 mg/mL and related compounds at 10 $\mu\text{g/mL}$, UV at 220 nm.

Compound	Retention time (min)				Retentivity factor (k*)				USP resolution (Rs)			
	UPLC H-Class (1.7 µm)	Arc (2.5 µm)	Arc HPLC (3.5 µm)	Alliance iS HPLC (3.5 µm)	UPLC H-Class (1.7 µm)	Arc (2.5 µm)	Arc HPLC (3.5 µm)	Alliance iS HPLC (3.5 µm)	UPLC H-Class (1.7 µm)	Arc (2.5 µm)	Arc HPLC (3.5 µm)	Alliance iS HPLC (3.5 µm)
Uracil	0.513	0.747	1.141	1.134	0.9	1.0	1.0	1.0	n/a	n/a	n/a	n/a
Rel comp-D	0.627	0.955	1.407	1.385	1.3	1.5	1.5	1.4	3.3	4.6	4.2	3.1
Uridine	0.802	1.242	1.921	1.919	2.0	2.3	2.4	2.4	4.4	5.2	6.2	5.5
Ribavirin	0.934	1.455	2.265	2.261	2.5	2.8	3.0	3.0	2.7	3.1	3.2	2.9
Rel comp-A	2.840	4.269	6.766	6.735	9.5	10.1	10.9	10.8	41.0	49.2	48.5	40.4

Table 2. Separation parameters for method scaling and migration between systems. UV at 220 nm.

System Suitability

Performance of the method on each system was assessed by measuring repeatability of six replicate injections of a standard solution containing 100 µg/mL of ribavirin API with 10 µg/mL related compounds. The peak areas and retention time repeatability generated by the ACQUITY Arc, Arc HPLC, and Alliance iS HPLC systems were excellent and in agreement with results acquired on the ACQUITY UPLC H-Class PLUS System (Figure 4).

ACQUITY UPLC H-Class PLUS (1.7 μm , 2.1 \times 50 mm)

System Suitability Sample SetId: 10420 Result SetId: 14122 Channel Name: 220				
	Name	# of inj.	USP Rs (Ave)	% RSD of RT %RSD of PeakAreas
1	Uracil	6		0.15 0.18
2	Ribavirin RC-D	6	3.3	0.14 0.19
3	Uridine	6	4.7	0.12 0.37
4	Ribavirin	6	2.7	0.10 0.22
5	Ribavirin RC-A	6	40.7	0.04 0.85

ACQUITY Arc (2.5 μm , 3.0 \times 75 mm)

System Suitability Sample SetId: 13558 Result SetId: 13867 Channel Name: 2998 Ch1 220nm@4.8nm				
	Name	# of inj.	USP Rs (Ave)	% RSD of RT %RSD of PeakAreas
1	Uracil	6		0.08 0.15
2	Rel comp-D	6	4.6	0.05 0.14
3	Uridine	6	5.2	0.14 0.20
4	Ribavirin	6	3.1	0.16 0.25
5	Rel comp-A	6	49.0	0.01 0.62

Arc HPLC (3.5 μm , 4.6 \times 100 mm)

System Suitability Sample SetId: 14752 Result SetId: 15615 Channel Name: 2998 Ch1 220nm@4.8nm				
	Name	# of inj.	USP Rs (Ave)	% RSD of RT %RSD of PeakAreas
1	Uracil	6		0.22 0.51
2	Rel Comp. D	6	4.2	0.20 0.15
3	Uridine	6	6.2	0.39 0.86
4	Ribavirin	6	3.2	0.47 0.38
5	Rel Comp. A	6	49.0	0.11 0.67

Alliance iS HPLC (3.5 μm , 4.6 \times 100 mm)

System Suitability Sample SetId: 11732 Result SetId: 14412 Channel Name: Alliance iS TUVChA				
	Name	# of inj.	USP Rs (Ave)	% RSD of RT %RSD of PeakAreas
1	Uracil	6		0.02 0.27
2	Rel Comp. D	6	3.1	0.04 0.23
3	Uridine	6	5.4	0.05 0.60
4	Ribavirin	6	2.9	0.05 0.39
5	Rel Comp. A	6	40.5	0.01 1.04

Figure 4. System suitability results (n=6) for method scaling and migration. UV at 220 nm.

Assay Results for Related Compounds

To demonstrate performance of the method for assay analysis of related compounds content (% impurity) an API sample solution was spiked with related compounds and run on different systems with the appropriate column dimensions. The assay results were calculated by comparing the peak area of each related compound to the area of the API, performed using Empower Software. The assay results generated by the ACQUITY Arc, Arc HPLC, and Alliance iS HPLC systems were in agreement with the results produced on the ACQUITY UPLC H-Class PLUS System (Figures 5). The relative retention times (RRT values), calculated using Empower Software by comparing retention of each related compound to the ribavirin API, were comparable across systems.

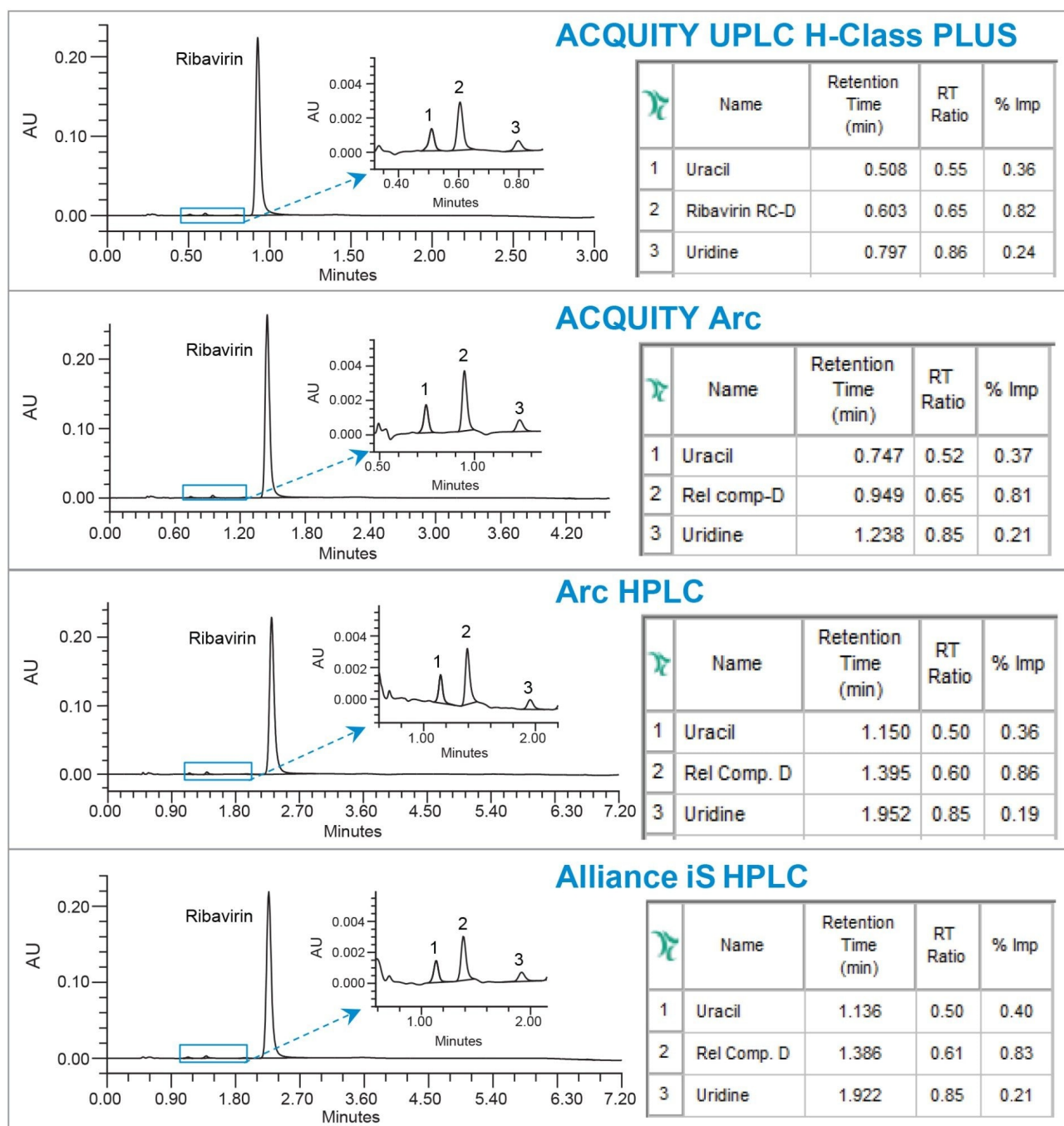


Figure 5. Assay results for related compounds content (% impurity) for method scaling and migration across systems. Ribavirin drug sample solution spiked with related compounds, UV at 220 nm.

Conclusion

A HILIC method for the analysis of ribavirin and related compounds was successfully scaled from 1.7 μm to 2.5, and 3.5 μm particle columns and migrated from an ACQUITY UPLC H-Class PLUS System to higher dispersion LC Systems, including ACQUITY Arc, Arc HPLC, and Alliance iS HPLC. The target systems generated equivalent chromatographic separation, system suitability, and assay results compared to the data produced on the ACQUITY UPLC H-Class PLUS System.

System characteristics including dwell and extra-column volumes can impact the quality of the chromatographic performance and must be accounted for to increase the success of method scaling and migration across different LC platforms.

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