

IMPACT OF INSTRUMENT CHARACTERISTICS ON THE METHOD MIGRATION OF A CHALLENGING USP ASSAY

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INTRODUCTION

USP monographs are designed to provide the user with the information needed to execute a given test. These monographs are used by scientists for a wide variety of reasons including potency and purity analysis, identification tests, and limit tests. In the case of LC chromatographic assays, the monograph will define the basic system suitability criteria needed to ensure the system is performing at a level required for the analysis. In some cases the same monograph can be analyzed on different LC systems, producing slightly different results but still meeting the system suitability requirements. This variability can be due to differences in system design, from the solvent managers, injectors to column heating and detectors. For example, binary and quaternary pumps are designed differently. These system configurations, while compliant, may not be optimal for every monograph, impacting system suitability results. This can be especially true of methods with challenging conditions or method conditions that are outside of typical operating range of a system.

The USP monograph azithromycin organic impurities is one such example. This monograph includes a long, shallow mobile phase gradient (0.5%B/column volume) relatively high salt content (12 mM), and a low wavelength (210 nm). All of these conditions combine to result in system suitability criteria that can be impacted by poor system performance. To assess the impact of system on the method, the organic impurities monograph was tested on multiple chromatographic systems, each with slight different design and instrument characteristics

METHODS

All samples and standards were prepared as described in the USP monograph with diluent of 7:6:7 methanol/acetonitrile/solution C where solution C was 1.73 mg/mL monobasic ammonium phosphate at pH 10.0. System suitability standard was prepared at 0.0165 mg/mL USP azithromycin related compound F and 0.027 mg/mL desosaminylazithromycin in diluent described above. Standard was prepared at 0.086 mg/ml azithromycin in diluent.

All batches consisted of six injections each of the system suitability standard and six injections of azithromycin standard.

Method Conditions	
Detector	TUV
Wavelength	210 nm
Column(s)	XBridge™ C18, 250 x 4.6 mm 5µm Column (p/n: 186003117)
Column Temp	60 °C
Sample Temp	5 °C
Injection Volume	50 µL
Flow Rate	1 mL/min
Mobile Phase A	1.8 mg/mL Sodium Phosphate Dibasic, pH 8.9
Mobile Phase B	3:1 Acetonitrile/Methanol
Needle Wash*	50:50 Acetonitrile:Water
Seal Wash*	10:90 Acetonitrile:Water
Gradient*when applicable	See Below

Gradient Table		
Time	A%	B%
0	50	50
25	45	55
30	40	60
80	25	75
81	50	50
93	50	50

Instruments	
Arc™ Premier HPLC System (Binary Pump)	
Alliance iS™ HPLC System (Quaternary Pump)	
Vendor X HPLC (Quaternary Pump)	
Vendor Y HPLC (Binary Pump)	
Vendor Z HPLC (Quaternary Pump)	

RESULTS AND DISCUSSION

Performance Comparison of HPLC Systems

The USP monograph has two system suitability requirements:

- peak to valley ratio no less than 1.4 in the system suitability standard
- azithromycin tailing between 0.8-1.5 in the azithromycin standard.¹

To compare the performance of HPLC systems the analysis was performed on the Alliance iS HPLC system and a legacy LC system. Slight differences in chromatography were observed with baseline resolution observed on the Alliance iS HPLC System while the legacy HPLC did not produce baseline resolution. A number of system characteristics could account for these differences, including extra-column dispersion, column temperature differences or other factors affecting selectivity.

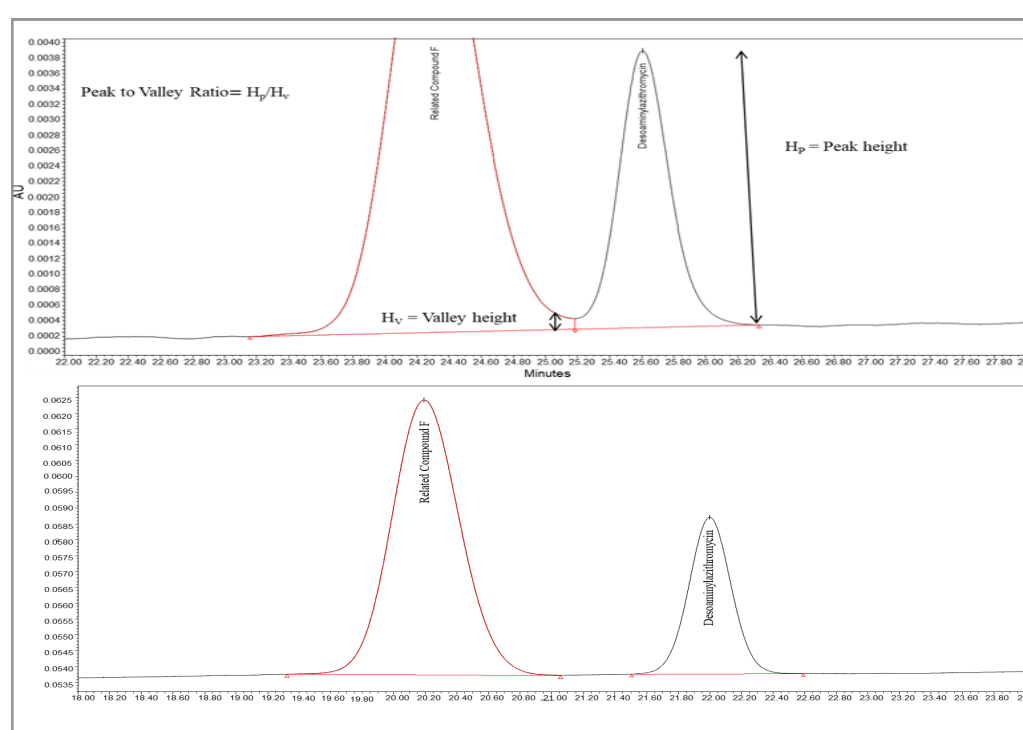


Figure 2 System suitability standard analyzed on Vendor X HPLC System (top) Alliance iS™ HPLC System (bottom). For Vendor X System, values used for peak to valley ratio between related compound F and desosaminylazithromycin are shown. Analyses were performed on both systems using the same column. Alliance iS™ HPLC System produced baseline resolution between analytes.

Peak to valley ratio is a resolution calculation typically used when peaks are coeluting or not baseline resolved. As seen in Figure 2, baseline resolution was not observed for the system suitability peaks on Vendor X HPLC system. In contrast, baseline resolution between on the Alliance iS HPLC System. As a result the peak to valley ratio cannot be calculated as there is no valley height between the two peaks.

When comparing the other system suitability criteria, the Alliance iS HPLC System produced similar peak tailing values when compared to Vendor X HPLC (Table 2).

System Suitability	Systems	
	Alliance iS HPLC System	Vendor X HPLC System
Azithromycin USP Tailing	1.05	1.09
Desosaminylazithromycin Peak to Valley Ratio	> 1.4 (Baseline Resolved)	47

Table 2. Tabulated system suitability results for the Alliance iS™ HPLC System and Vendor X HPLC System.

Whether due to dwell volume, gradient delivery or temperature effects, it is not uncommon for retention time differences to be observed across systems. While this was the case for the two analysis of azithromycin on the two systems, relative retention times were more comparable. The relative retention time as described in the monograph (0.51 for related compound F and 0.54 for desosaminylazithromycin) provides better guidance to identify peaks.

Alliance iS HPLC System		
	Retention Time	Relative Retention Time
Rel Compound F	20.20	0.45
Desosaminylazithromycin	22.0	0.49
Azithromycin	45.02	N/A

Vendor X HPLC		
	Retention Time	Relative Retention Time
Rel Compound F	24.1	0.48
Desosaminylazithromycin	25.41	0.51
Azithromycin	50.34	N/A

Table 3: Comparison of retention time and relative retention time for system suitability peaks on Alliance iS™ HPLC System and Vendor X System.

The long, shallow mobile phase gradient in this method can be difficult for systems to accurately and precisely deliver, resulting in shifting retention times and thus higher %RSD values. The Alliance iS HPLC System produced more consistent retention time reproducibility compared to Vendor X HPLC System.

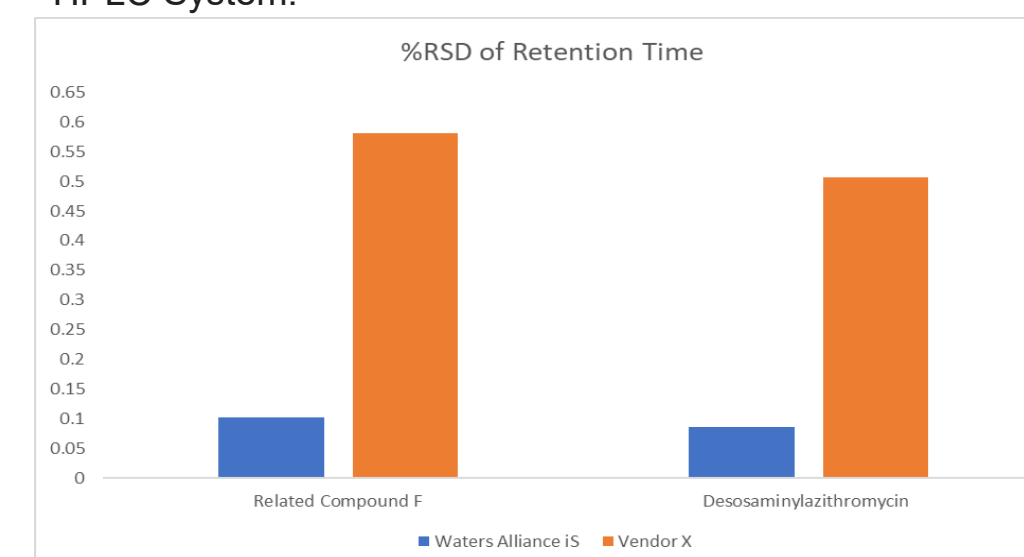


Figure 3. %RSD values for Related Compound F and Desosaminylazithromycin retention times for the Alliance iS™ HPLC System and Vendor X systems (n=6 injections).

Performance Comparison of Binary and Quaternary Mid-Pressure HPLC Systems

The same analysis was compared on both a quaternary and a binary mid-pressure (>9,000 psi) systems. Due to the challenging gradient of 0.5%/column volume, a binary solvent manager will typically produce more precise retention times. This is due to the presence of two separate pump heads on the binary pump with one delivering mobile phase A and the other mobile phase B. Alternatively a quaternary system relies on the gradient proportioning valve to adjust the mobile phase composition. Though there are drawbacks to using a binary system, like limited solvent mixing (two at a time) and increased cost, a binary system can be ideal for methods involving a shallow mobile phase gradient.

While all systems met system suitability criteria (data not shown), the binary solvent managers showed comparable or improved results when compared to a quaternary system. This trend was observed for both Waters and competitors binary systems.

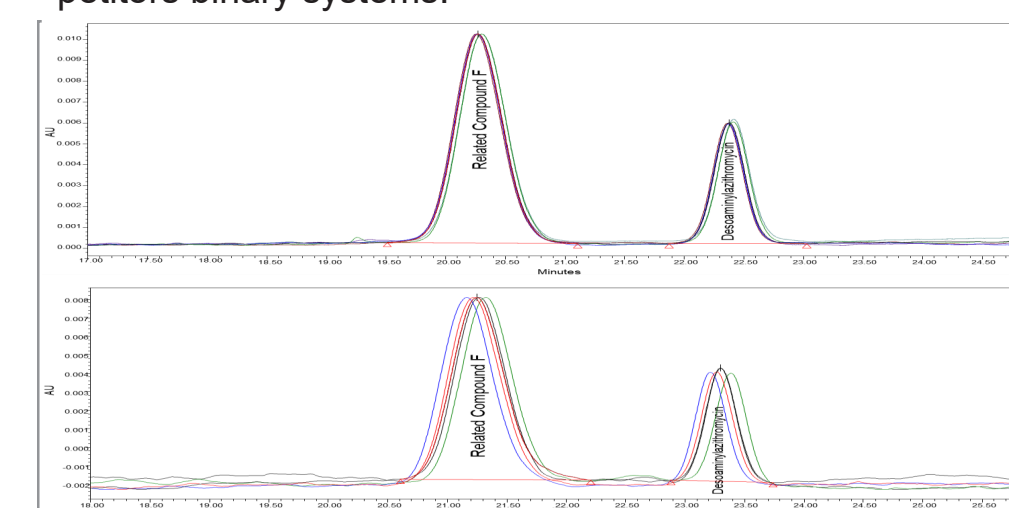


Figure 4. UV overlay of n=6 injections of the system suitability standard. Top: Arc™ Premier HPLC System (Binary pump). Bottom: Vendor Z (Quaternary pump).

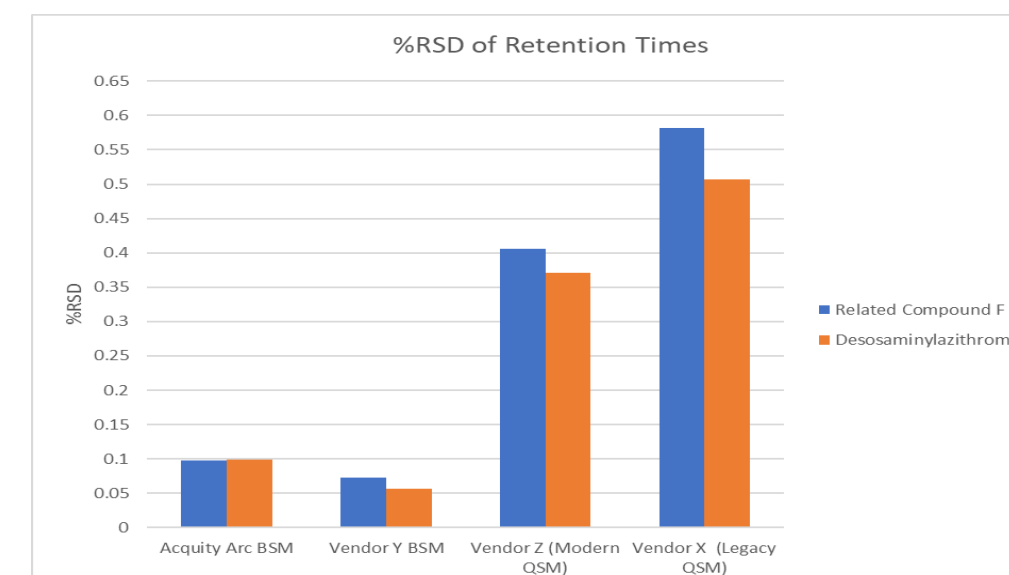


Figure 5. %RSD values for Related Compound F and Desosaminylazithromycin retention times for the Arc™ Premier HPLC System (Binary pump), Vendor Y (Binary Pump), Vendor Z (Modern Quaternary pump), and Vendor X (Legacy Quaternary)

For this method, the improvement in retention time precision for binary systems was significant (4-6x improvement) as compared to quaternary systems; however the Alliance iS HPLC System produced comparable retention time %RSD values as binary systems (Fig 3). Thus, the Alliance iS HPLC System performance under high salt conditions was significantly improved over other quaternary systems.

Impact of High Salt on HPLC Performance

Throughout the course of this study, some instruments showed atypical high %RSD values for retention time repeatability. Investigation into the behavior led to analysis of the pressure traces of each run. Analysis showed system pressure drops throughout the sample set. In at least one instance, another vendor's quaternary system saw pressure dips sporadically throughout injections and the sample set. These dips correlated to regions of the chromatogram where the organic solvent content was high in relation to the aqueous buffer, suggesting the dips may have been the result of salt crashing out of solution during mixing. The pressure dips could also be indicative of any number of things, but suggest salt formation is impacting the system performance. In most cases, the system was able to recover and continue but retention time reproducibility suffered.

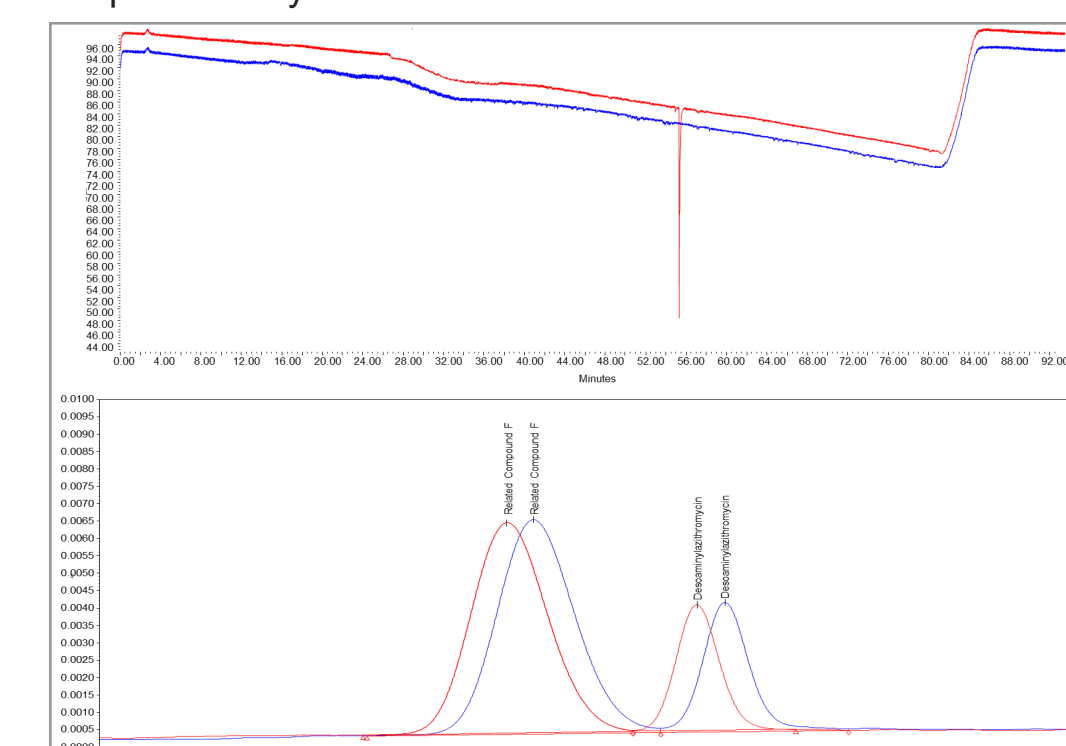


Figure 6. Subsequent injections showing shifting retention times. Top: System pressure showing a drop in pressure (red trace), and the subsequent run (blue trace). Pressure traces are staggered. Bottom: UV signal showing a retention time shift.

CONCLUSIONS

- Method conditions, outside the typical operating range of a system, may pose unique challenges for method migration and meeting system suitability criteria.
- For the method tested - with high salt mobile phases and long shallow gradients- binary pumps typically provided greater retention time precision.
- The Alliance iS HPLC System produced more reproducible retention times and greater USP resolution for critical pair as compared to other legacy quaternary HPLC Systems. The retention time precision was comparable to the binary systems.
- System characteristics should be considered when executing methods, especially for those with long shallow gradients or when atypical method conditions are used.

References

1. United States Pharmacopeia (2022) USP Monographs Azithromycin Organic Impurities, USP-NF. Rockville, MD:USP DOI:https://doi.org/10.31003/USPNF_M6740_05_01