# CONSIDERATIONS FOR METHOD MIGRATION BETWEEN HPLC PLATFORMS IN THE ANALYTICAL LABORATORY

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## INTRODUCTION

**In today's global economy, companies constantly strive to bring their commodities to market before other competitors. Analytical chromatography laboratories, therefore, must implement efficient procedures to accurately evaluate their products. Whether analyses are performed using the same instrumentation or diverse chromatographical platforms in different laboratories, well-characterized instrument attributes aid in the efficient method migration between systems.**

**Successful migration of methods between HPLC systems is a multiple step process involving an understanding of how differences in instrumentation affect the separation and development of a control strategy to mitigate these differences. System dwell volume and extra column dispersion both impact the chromatographic separation and should be characterized when migrating methods between systems.**

**This study presents the steps taken in the migration of methods from two legacy HPLC systems (system 1 = Waters Alliance™ System; system 2 = system Y from a different vendor) to a modern HPLC system (Waters Alliance™ iS HPLC System).**

### Dwell Volume

Dwell volume is the volume between the point of mixing and the column inlet. It is impacted by tubing length and internal diameter, mixers, and valves in the fluidic path before the head of the column. Differences in dwell volume result in variation of retention times between systems. Selectivity may also be affected.

The impact of the different dwell volumes of each system on retention time was assessed utilizing a gradient method for the separation of a generic test mix. Differences in dwell volume were then compensated for by adjusting the gradient start relative to the injection.

### Extra Column Dispersion

Extra column dispersion is the extra column volume in an HPLC system. The primary sources of extra column dispersion are tubing, heat exchangers, connectors and fittings and the detector flow cell. Because modern HPLC systems are highly configurable, significant differences in extra column dispersion may exist between systems.
Extra column dispersion affects peak width, resolution and the overall efficiency of a separation. Gradient separations which focus peaks at the head of the column are impacted to a lesser extent by extra column dispersion then isocratic separations.

To illustrate the effects of extra column dispersion, an isocratic separation, the USP fluconazole organic impurities method, was run on each of the systems.

## METHODS

### Dwell Volume

| Dwell Volume Characterization | |
|---|---|
| **Mobile Phase A** | Water |
| **Mobile Phase B** | 10 mg/mL Caffeine in Water |
| **Flow Rate** | 1.00 mL/min |
| **Run Time** | 40 minutes |
| **Gradient Table** | Time / %A / %B / Curve<br>0.00 / 100.0 / 0.0 / Initial<br>5.00 / 100.0 / 0.0 / 6<br>25.00 / 0.0 / 100.0 / 6<br>30.00 / 100.0 / 0.0 / 11<br>40.00 / 100.0 / 0.0 / 11 |
| **Injection Volume** | 0.0 µL |
| **Column Temperature** | 30.0ºC |
| **Column** | Zero Dead Volume Union |
| **Detector** | UV: λ = 273 nm; 20 Hz |

| Generic Test Mix | |
|---|---|
| **Mobile Phase A** | 0.1% Formic Acid in Water |
| **Mobile Phase B** | 0.1% Formic Acid in Acetonitrile |
| **Flow Rate** | 1.00 mL/min |
| **Run Time** | 14 minutes |
| **Gradient Table** | Time / %A / %B / Curve<br>0.00 / 80.0 / 20.0 / Initial<br>3.50 / 80.0 / 20.0 / 6<br>8.75 / 10.0 / 90.0 / 6<br>10.50 / 10.0 / 90.0 / 6<br>10.54 / 80.0 / 20.0 / 6<br>14.00 / 80.0 / 20.0 / 6 |
| **Injection Volume** | 10.0 µL |
| **Column Temperature** | 45.0ºC |
| **Sample Temperature** | 10.0ºC |
| **Column** | Waters XBridge™ C18 Column 4.6 x 50 mm, 3.5 µm |
| **Detector** | UV: λ = 254 nm; 20 Hz |

### Extra Column Dispersion

| Extra Column Dispersion Characterization | |
|---|---|
| **Mobile Phase A** | Water |
| **Mobile Phase B** | Acetonitrile |
| **Flow Rate** | 1.00 mL/min |
| **Run Time** | 1.00 minutes |
| **Gradient Table** | Time / %A / %B / Curve<br>0.00 / 70.0 / 30.0 / Initial<br>1.00 / 70.0 / 30.0 / 6 |
| **Injection Volume** | 0.0 µL |
| **Column Temperature** | Ambient |
| **Sample Temperature** | Ambient |
| **Column** | Zero Dead Volume Union |
| **Detector** | UV: λ = 273 nm; 40 Hz |

| USP Fluconazole Organic Impurities | |
|---|---|
| **Mobile Phase** | 80:20 Water:Acetonitrile |
| **Flow Rate** | 0.50 mL/min |
| **Run Time** | 20.00 minutes |
| **Gradient Table** | Time / %A / Curve<br>0.00 / 100.0 / Initial<br>20.00 / 100.0 / 6 |
| **Injection Volume** | 20.0 µL |
| **Column Temperature** | 40.0ºC |
| **Sample Temperature** | 15.0ºC |
| **Column** | Waters XSelect™ HSS T3 Column: 4.6 x 150 mm, 3.5µm |
| **Detector** | UV: λ = 260 nm; 10 Hz |

## RESULTS & DISCUSSION

### Instrument Characterization

To understand the impact of instrument characteristics on our studies, each system's dwell volume and extra column dispersion were measured.

***Dwell volume measurements***

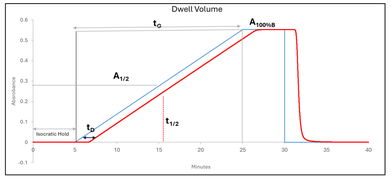


*Figure 1. Dwell volume measurements were performed on each system.*

*Calculations were performed as follows:*

1. *Calculate Absorbance at 50%: $A_{1/2} = (A_{100\%B} - A_{0\%B})/2$*
2. *Determine retention time for $A_{1/2}$, which equals $t_{1/2}$.*
3. *Calculate gradient delay in time ($t_D$) using $t_D = t_{1/2} - 1/2(t_G)$ where $t_G$ = total gradient time.*
4. *Calculate adjusted (for isocratic hold) gradient delay time: $t_{Dadj} = t_D$ - isocratic hold time*
5. *Convert $t_{Dadj}$ to dwell volume . $V_D = t_{Dadj} * $ flow rate.*

| System | Dwell Volume ($V_D$) (mL) |
|---|---|
| Alliance System | 1.15 |
| System Y HPLC | 1.44 |
| Alliance iS HPLC System | 1.59 |

***Extra-column dispersion measurements***

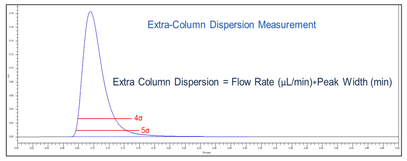


*Figure 2. Extra-column dispersion measurements were made using the Empower™ 3 Chromatography Data System. Results were obtained by multiplying the peak width at 13.4% height (4 σ) and 4.4% peak height (5 σ) by the flow rate.*

| System | Extra Column Dispersion @ 4σ | Extra Column Dispersion @ 5σ |
|---|---|---|
| Alliance System | 50.2 µL | 68.5 |
| System Y | 40.0 µL | 55.2 |
| Alliance iS HPLC System | 26.6 µL | 36.4 |

## RESULTS & DISCUSSION

### Impact of Dwell Volume::

The impact of dwell volume on gradient separations was demonstrated by running the generic test mix on each system. As expected, due to variation in dwell volume between the systems differences in retention times are seen. Relative retention times however are the same across the systems.

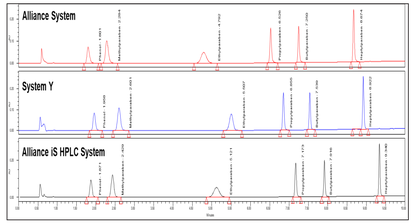


Figure 3. *Chromatogram of the generic test mix on each system.*

| | RRT: Alliance System | RRT: System Y | RRT: Alliance iS HPLC System |
|---|---|---|---|
| Phenol | 0.21 | 0.22 | 0.20 |
| Methylparaben | 0.26 | 0.29 | 0.26 |
| Ethylparaben | 0.55 | 0.62 | 0.55 |
| Propylparaben | 0.75 | 0.77 | 0.77 |
| Butylparaben | 0.84 | 0.84 | 0.85 |
| Heptylparaben | 1.00 | 1.00 | 1.00 |

Variation in retention times that result from dwell volume differences between systems may be mitigated by adjusting the gradient start relative to the injection. Such adjustments for dwell volume differences are allowed per USP <621>.
The generic test mix was run on the Alliance iS HPLC System using a method compensated for the differences in dwell volume. The resulting chromatogram show retention time shifts aligning the chromatograms to that obtained on the legacy system.

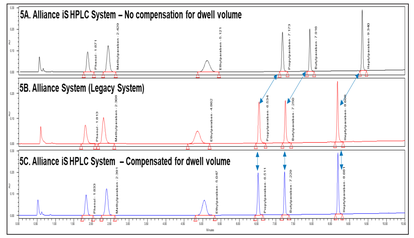


*Figure 4. Chromatograms of the generic test mix.*

*Figures 4A & 4B. The retention times obtained on the Alliance iS HPLC System and the legacy system do not align.*

*Figures 4B & 4C. The method was compensated for the difference in dwell volume between the legacy system and the Alliance iS HPLC System. The compensated method produced retention times that align with that obtained on the legacy Alliance System.*

## RESULTS & DISCUSSION

### Impact of Extra Column Dispersion::

The impact of extra column dispersion on an isocratic separation was seen by running the USP Fluconazole Organic Impurities method on each system. The results show that the system with the greatest amount of dispersion had the lowest peak efficiencies (USP Plate Count). The results also demonstrate that extra column dispersion affects Resolution especially for early eluting peaks.

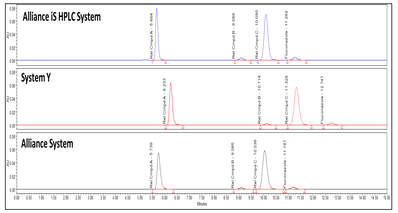


Figure 5. *Representative Chromatograms of the Fluconazole Organic Impurities Standard Solution.*

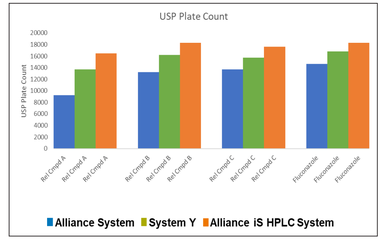


*Figure 6. USP Plate Count. The Alliance iS HPLC System which has the lowest extra-column dispersion has the highest plate count.*

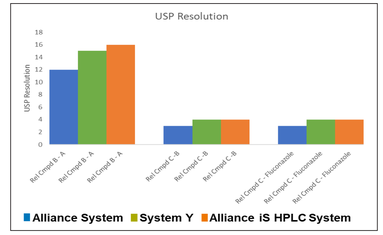


*Figure 7. USP Resolution. Although each system met the system suitability Resolution requirement, the lowest dispersion system showed an increase in Resolution for early eluting peaks.*

## CONCLUSION

**Well-characterized instrument attributes aid in the efficient method migration between systems. Methods were successfully migrated from two legacy HPLC systems (system 1 = Waters Alliance System; system 2 = System Y from a different vendor) to a modern HPLC system, the Waters Alliance iS HPLC System.**

- **Dwell volume and extra column dispersion were characterized on each system.**
- **The impact of dwell volume and extra column dispersion on chromatographic separations were determined.**
- **With a gradient method, differences in dwell volume between systems resulted in retention time variation. These differences were compensated for by adjusting the gradient start.**
- **An isocratic method was utilized to demonstrate how differences in extra column dispersion between systems affect peak efficiency and resolution. The Alliance iS HPLC System which has the lowest extra column dispersion of the systems showed the highest plate count and improved resolution of early eluting peaks.**

**The study demonstrated a proactive and successful strategy for migration of methods from legacy systems, and the performance benefits of keeping instrumentation assets up to date.**

*Figure 8. Alliance iS HPLC System*

**References**

1. P. Hong et al., *Dwell Volume and Extra Column Volume: What Are They and How Do They Impact Method Transfer?* (Waters Corp., 2018).
2. United States Pharmacopeia (2023). *USP Monographs, Fluconazole*. USP-NF. Rockville, MD:USP. DOI: https://doi.org/10.31003/USPNF_M33240_03_01

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