

## METHOD TRANSFER ACROSS HPLC PLATFORMS

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### INTRODUCTION

Within the pharmaceutical analysis space, it has become commonplace to transfer methods across HPLC platforms on a routine basis. This is for a variety of reasons including the global expanse of the industry with labs in different locations having different instrumentation available, the use of contract labs which may have different instrumentation, and upgrading systems to take advantage of modern technologies. In many instances, methods can be successfully transferred with little or no intervention, and resulting chromatographic equivalence and performance metrics are achieved. However, when method transfer does not produce expected results, it can be difficult to identify the root cause and make the necessary adjustments. Additionally, when methods are transferred within a regulated environment, allowable adjustments, either to the method conditions or the instrument hardware, will vary and may require some amount of re-validation or re-qualification. For these reasons and others, method transfer can be a burdensome yet necessary task.

This presentation will review a method transfer example using hesperidin, which is used in traditional Chinese medicine as a cough suppressant/expectorant and is sourced from citrus fruits, specifically tangerine. In this example, the impact of hardware design differences will be explored. Success of the method transfer will be determined based on performance criteria, including retention time/area precision and peak shape.

### METHODS

HPLC Systems:

Legacy Alliance™ HPLC



Alliance iS HPLC System



Methods:

Hesperidin (based off Chinese Pharmacopeia Method):

#### Hesperidin Conditions

|                    |   |
|--------------------|---|
| Mobile Phase       | 67:33 0.1% phosphoric acid in water:acetonitrile (v:v)      |
| Flow Rate          | 1 mL/min  |
| Column             | Waters XBridge™ BEH™ C18 4.6 x 250 mm, 5 µm; p/n: 186003117 |
| Column Temperature | 25 °C   |
| Sample Temperature | 8 °C  |
| Injection Volume   | 10 µL   |
| Detection          | 283 nm; 10 Hz   |

Sample Preparation:

Hesperidin (Sigma Aldrich) was prepared to a final concentration of 30 µg/mL in methanol

Dispersion Evaluation Conditions:

#### Dispersion Conditions

|                  |                               |
|------------------|-------------------------------|
| Mobile Phase     | 30:70 water:acetonitrile      |
| Flow Rate        | 1 mL/min                      |
| Column           | Union used in place of column |
| Injection Volume | 1 µL                          |
| Detection        | 273 nm; 40 Hz                 |

Sample Preparation:

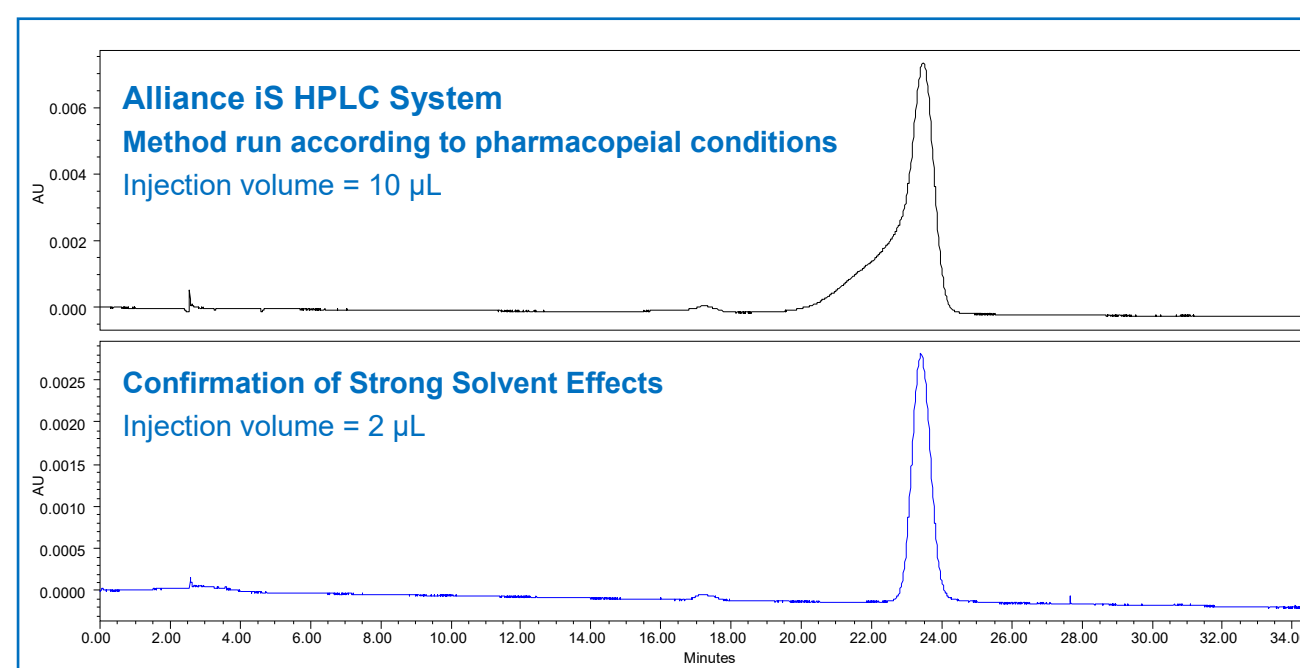
Caffeine was prepared at a concentration of 0.26 mg/mL in 90:10 water:acetonitrile.

Additional LC Hardware— Additional volume was added after injection using various approaches, which included larger ID tubing and two sample loops of varying shapes:

- Alliance iS HPLC System High Flow Kit— p/n: 205002525
- Extension Loop, 50 µL— p/n: 430002012
- Bio Extension Loop, 50 µL— p/n: 430002597

### RESULTS & DISCUSSION

The hesperidin method was initially executed on the legacy Alliance HPLC System. When the method was transferred to the Alliance iS HPLC System, the hesperidin peak showed significant fronting. The isocratic method uses elution conditions that are primarily aqueous (67:33 0.1% phosphoric acid in water: acetonitrile), while the injection solvent is 100% methanol. While not ideal for the injection solvent to be higher in elution strength, it may be necessary due to solubility issues for example. If the injection solvent is sufficiently strong and there is not enough mixing volume post sample injection, strong solvent effects can result in distorted peak shapes. Figure 1 shows the impact of strong solvent effects on the analysis of hesperidin.



One way to determine if the peak shape distortion is due to the injection solvent strength is to decrease the injection volume. In this example, the smaller injection volume of 2 µL (which injects less organic) results in peak shape that is dramatically improved. When a method has been fully validated, it may not be possible or practical to reduce the injection volume due to regulations and potential negative impact of the method change such as decreased limit of detection or increased peak area RSDs. A change to the LC hardware, however, may be able to solve the problem without making any method adjustments.

Figure 1. Chromatograms obtained for hesperidin using the prescribed pharmacopeial conditions (top) and a reduced injection volume (bottom). The gaussian peak shape obtained with a reduced injection volume confirms strong solvent effect.

As previously stated, strong solvent effects occur when there is not a sufficient amount of mixing volume post-injection. When there is sufficient mixing, the strong solvent is effectively diluted and peak shape is symmetrical. In this case, the mixing volume is mainly determined by tubing dimensions. Many legacy HPLCs use relatively large tubing IDs, such as 0.010". Newer HPLCs often optimize tubing IDs to be smaller, which results in lower system dispersion. A lower dispersion can be advantageous for gaining chromatographic performance when using columns with smaller particles, however, when running legacy HPLC methods with large injection volumes (ideal for larger 5 µm particle columns), this lower dispersion can lead to strong solvent effects. One way to reduce strong solvent effects is to add additional volume post injection to aid in dilution of the strong injection solvent. This was done in two ways: 1) increase the tubing ID post injection (High Flow Kit) or 2) adding a loop post injection. In this example, two loops were evaluated, both with a volume of 50 µL, but with different inner diameters and lengths.

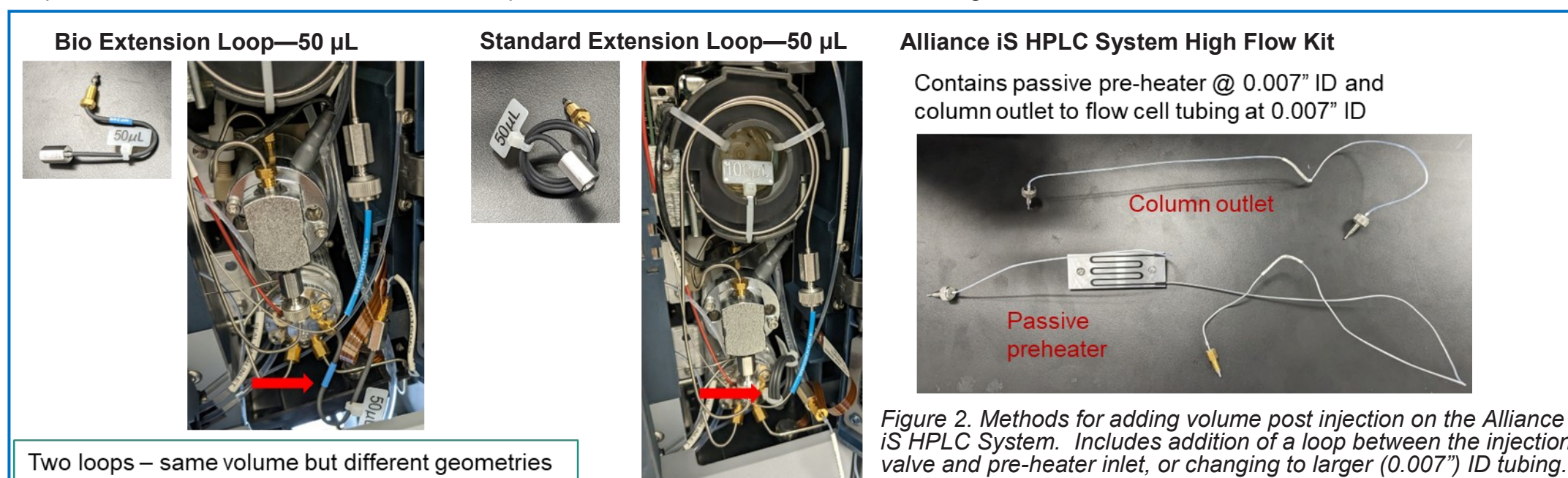


Figure 2. Methods for adding volume post injection on the Alliance iS HPLC System. Includes addition of a loop between the injection valve and pre-heater inlet, or changing to larger (0.007") ID tubing.

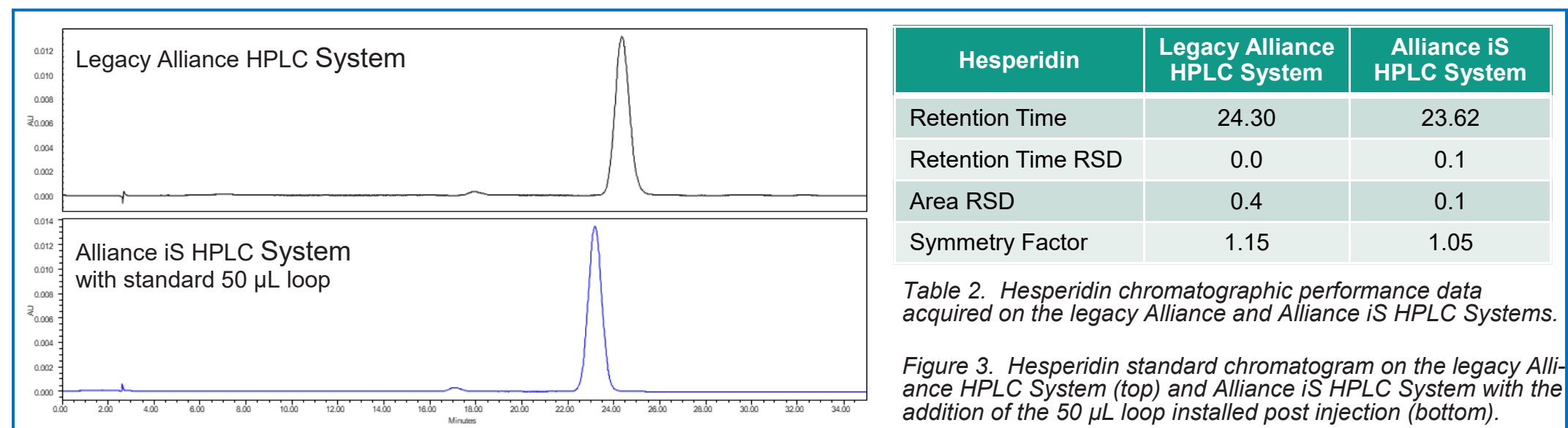
To determine the impact on dispersion of these methods for adding volume post injection, system dispersion was measured using caffeine injections with no column. In addition to measuring standard system dispersion on the Alliance iS HPLC System, dispersion was also measured using the optional 0.007" tubing, as well as two 50 µL loops of different geometries. The measured dispersion values (4 σ) are shown below.

|   | Dispersion µL<br>4 σ |
|---|----------------------|
| Alliance iS HPLC System - Standard Configuration                                  | 25.4                 |
| 50 µL Bio extension loop added  | 61.6                 |
| 50 µL Standard extension loop added   | 68.2                 |
| High Flow Kit Installed   | 34.1                 |
| Alliance iS HPLC System with High Flow Kit Installed & 50 µL Standard Mixer Added | 73.4                 |
| Legacy Alliance HPLC System (w/ passive pre-heater, 2489)                         | 77.5                 |

Most noticeable, the legacy Alliance HPLC System equipped with a passive pre-heater has a dispersion value 3x greater than the Alliance iS HPLC System. Addition of the larger ID tubing (High Flow Kit) only increased dispersion from ~25 µL to ~35 µL, which likely is not enough to resolve the significant peak fronting seen with hesperidin. Addition of a 50 µL loop increased dispersion to a level nearly commensurate with the Alliance HPLC System. Ultimately, additional volume was added by using the standard extension loop— this offered the easiest solution since only 1 part needed to be added to the system in contrast to addition of the high flow kit where 3 parts needed to be added/changed.

Table 1. Measured dispersion values using caffeine at 4σ (13.4% peak height).

Hesperidin was run again with the standard 50 µL loop installed between the injection valve and the pre-heater inlet tubing with the resulting chromatography shown below.



| Hesperidin         | Legacy Alliance HPLC System | Alliance iS HPLC System |
|--------------------|-----------------------------|-------------------------|
| Retention Time     | 24.30                       | 23.62                   |
| Retention Time RSD | 0.0                         | 0.1                     |
| Area RSD           | 0.4                         | 0.1                     |
| Symmetry Factor    | 1.15                        | 1.05                    |

Table 2. Hesperidin chromatographic performance data acquired on the legacy Alliance and Alliance iS HPLC Systems.

Figure 3. Hesperidin standard chromatogram on the legacy Alliance HPLC System (top) and Alliance iS HPLC System with the addition of the 50 µL loop installed post injection (bottom).

The addition of the 50 µL loop successfully eliminated the strong solvent effects initially seen on the Alliance iS HPLC System. The peak shape is now symmetrical, with slightly less tailing than was seen on the Alliance HPLC System. Absolute retention times were similar between the two systems. Finally, retention time and area RSDs were extremely low on both systems. This highlights the success of the method transfer from the legacy Alliance to Alliance iS HPLC System with no changes to the method when a small amount of mixing volume is added post injection.

### CONCLUSION

- Method transfer can be challenging when transferring across different LC system platforms that have varied instrument characteristics
- Methods which use a strong injection solvent (stronger than method initial conditions) can result in strong solvent effects
- Adding a small amount of volume post injection, such as the addition of a 50 µL loop, can successfully resolve strong solvent effects without the need for method adjustments

References: Xingsu Zhike Koufuye (Hesperidin)—ChP.2020.2083-2084.

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