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Application Note

Transfer of an HPLC Method from an Agilent 1100 Series LC System to an Alliance HPLC System

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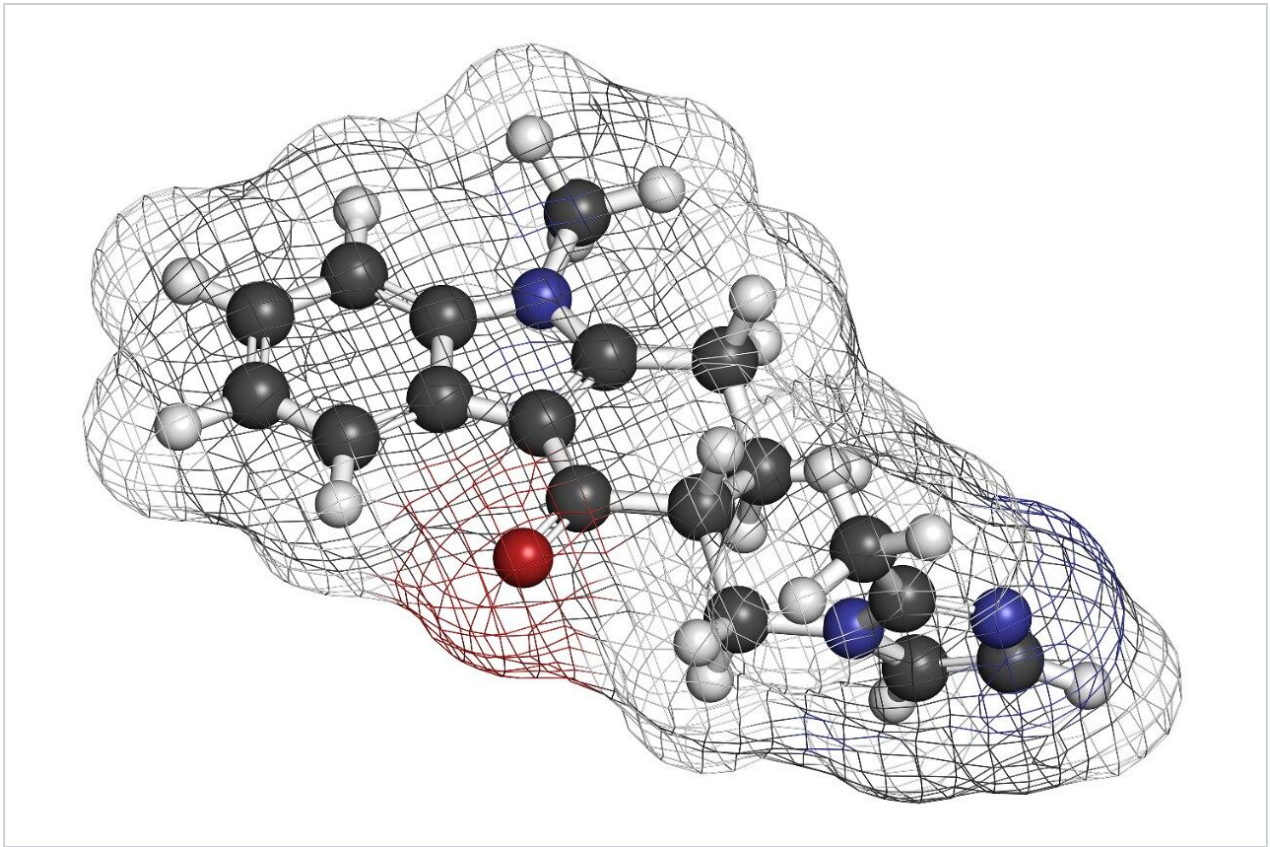

This is an Application Brief and does not contain a detailed Experimental section.

Abstract

This applicationn brief demonstrates the successful transfer of an HPLC method from an Agilent 1100 Series LC System to an Alliance HPLC System with minimal adjustments to the method.

Benefits

Seamless methods transfer of a challenging HPLC method from an Agilent 1100 Series LC System to an Alliance HPLC System.

Introduction

Ever-increasing globalization and consolidation of resources can result in a need to transfer HPLC methods across different laboratories. Though it is often preferred to perform analyses on the same type of instrumentation across these different locations, this is often not always possible. In these instances, there may be a need to transfer the method to an HPLC instrument from a different manufacturer. The design characteristics of each system can affect this transfer. These effects are often most apparent for complex gradient separations, in which the gradient delay of the system can alter the mobile phase composition at the sample injection. One approach to compensate for these variations involves making gradient adjustments based on differences in each system's dwell volume. This may be cumbersome and require additional testing of the instrumentation. However, if the instrumentations' dwell volumes are comparable, no changes to the method may yield acceptable results, and provide a more desirable approach.

Results and Discussion

In methods transfer, the preference is to meet established method criteria with the original method conditions, or without the need to make any adjustments. When transferring across similar instrumentation, such as from one HPLC to another HPLC system, the minimal difference in dwell volumes can increase the likelihood of meeting the required criteria. To evaluate one such example, a challenging separation was developed on an Agilent 1100 Series LC System and transferred to an Alliance HPLC System (Table 1).

Specifically the separation of a mixture of ten components was developed on a ZORBAX SB C_{18} RR 3.5 µm, 4.6 x 150 mm column using an Agilent 1100 Series LC System. The same column and mobile phases were transferred to the Alliance System with a 2998 PDA Detector with no adjustments made for differences in each system's dwell volume.

Agilent 1100 Series LC System		Alliance HPLC System	
Module	**Part number**	**Module**	**Part number**
Degasser	G1322A	e2695 Alliance	186269506
Quaternary Pump	G1311A		
Autosampler	G1313A		
Column Compartment	G1316A	Column Compartment	186179100
DAD detector	G1315B	998 PDA Detector	186299800

Table 1. Chromatography Data System (both): Empower3 FR2.

The results of the methods transfer produced separations with minimal differences in retention times across both HPLC instruments (Figure 1). Although the retention times were earlier on the Alliance HPLC System as compared to the Agilent 1100 Series LC System, the difference or deviation was within the desired variation of not more than 5%[1] (Table 2). Furthermore, the resolution of the critical pair was preserved.

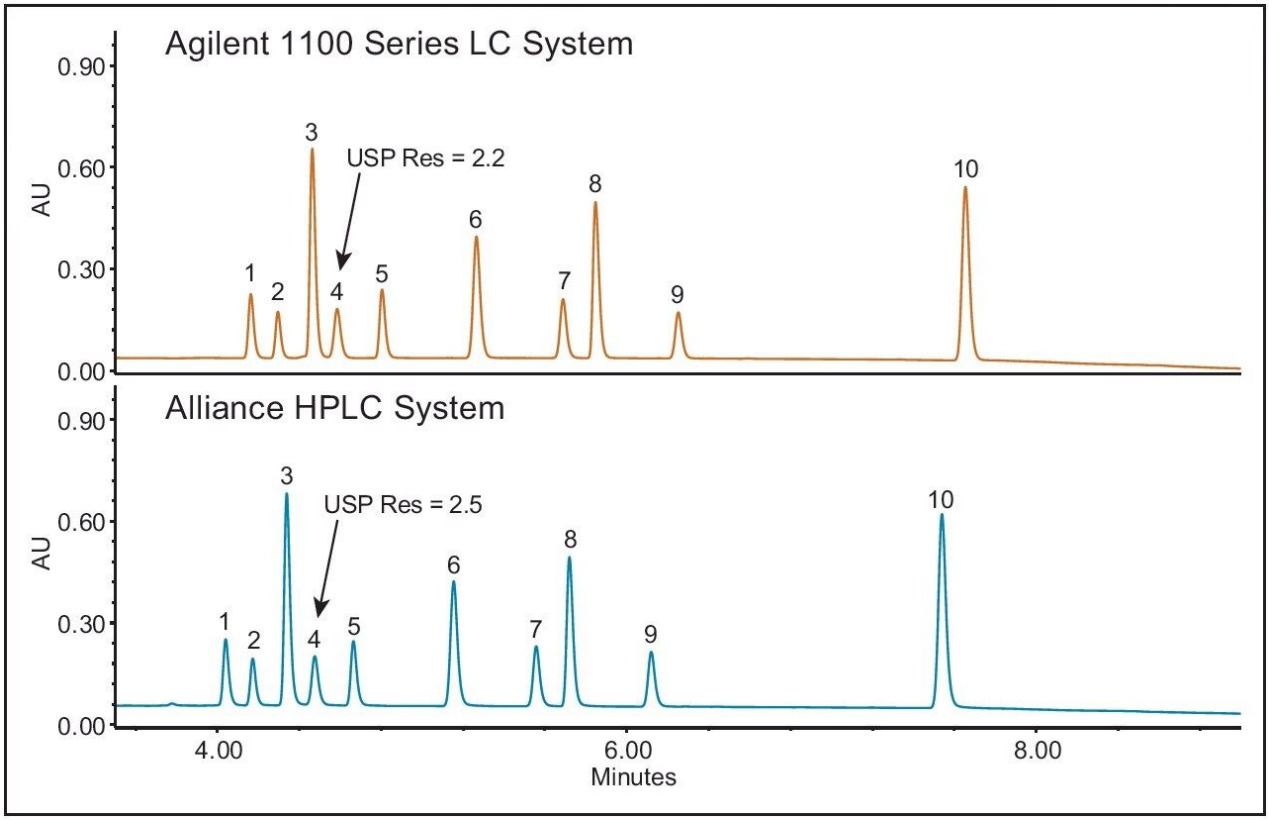

Figure 1. Separation of complex mixture, including ondansetron and related compounds, on both an Agilent 1100 LC Series System and an Alliance HPLC System. No adjustments were made to the gradient method on the Alliance HPLC System. Similar retention were observed for all compounds and the USP resolution of the critical pair was preserved.

Compound	Agilent 1100 Series LC System	Alliance HPLC System	% Deviation
Rel impurity A	4.17	4.03	3.19
Rel Impurity G	4.30	4.17	3.07
Ondansetron	4.47	4.33	2.98
Florifenicol	4.59	4.47	2.53
Buspirone	4.81	4.66	3.06
Coumarin	5.27	5.15	2.22
Rel Compound C	5.69	5.55	2.41
Protriptyline	5.85	5.71	2.34
Rel Compound D	6.25	6.11	2.21
Flavone	7.66	7.53	1.58

Table 2. Retention times observed on the Agilent 1100 Series LC System and the Alliance HPLC System. Method transfer was performed witout any adjustments to compensate for dwell volume differences between the two systems. The retention times were within 0.15 minutes or less than 3.5% difference on the Alliance HPLC System as compared to the Agilent 1100 Quaternary Series LC System. All values are average of six replicate injections.

However, it has been well understood that the gradient delay or dwell volume of each system can impact the retention time difference or deviation. In fact, "dwell volume adjustments" to LC methods allowed under USP guidelines.[2] Thus, to evaluate the impact of dwell volume differences the same experiment was repeated with gradient adjustments made for dwell volume differences. This process required:

- Measuring the dwell volume of each instrument[3]
- Calculating the gradient adjustment required for methods transfer

Using a previously described procedure,[3] the dwell volumes were measured for the specific systems. The dwell volume of the Agilent 1100 Series LC System (1.290 mL) was greater than that of the Alliance HPLC System (1.15 mL) (Figure 2). The Waters Column Calculator was then used to determine the gradient adjustments for methods transfer. Using the calculator, a gradient adjustment or delay for the method transfer of 0.09 min at 1.50 mL/min (0.14 mL) was used on the Alliance HPLC System to compensate for the differences in dwell volume. This was the only adjustment to the method.

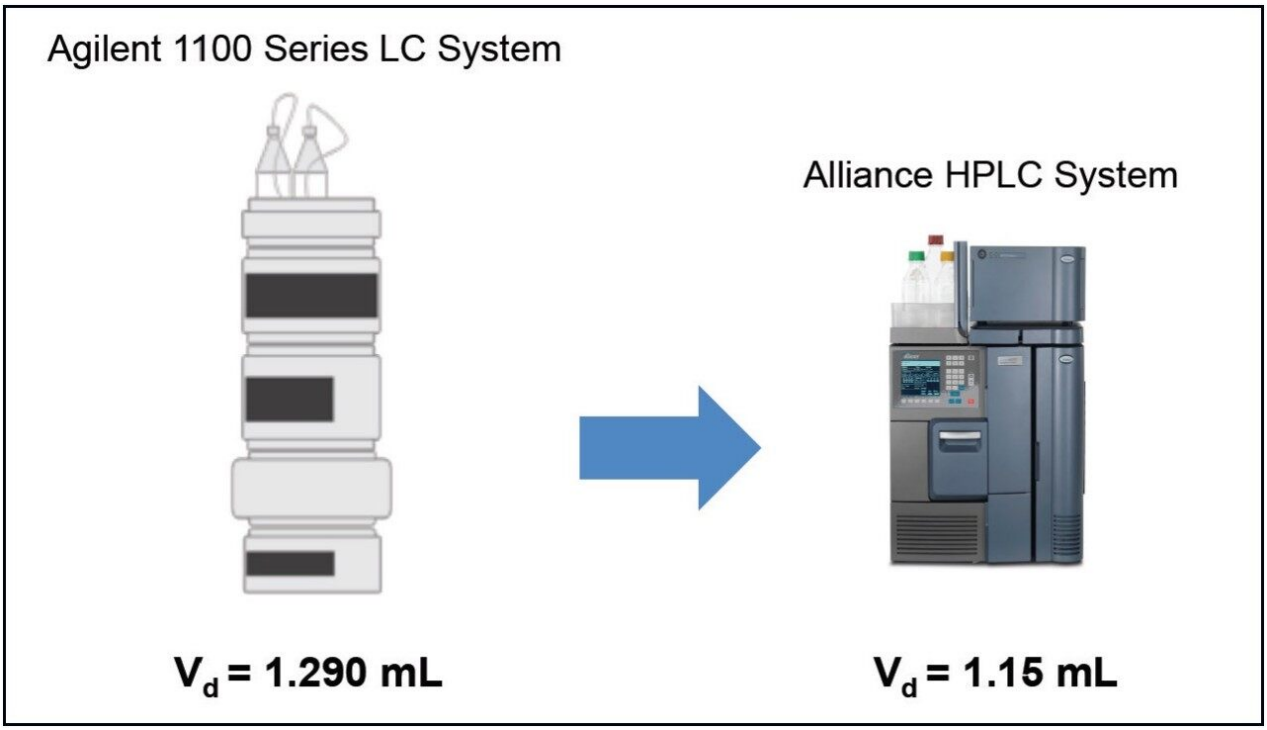

Figure 2. Dwell volume measurements were performed for both an Agilent 1100 Series LC System and an Alliance HPLC System as previously described.[3] Using these values, the difference in dwell volumes was calculated (0.140 mL) for methods transfer from the Agilent 1100 Series LC System to the Alliance HPLC System. This value was converted to time for adjustment of the gradient within guidleines.[2]

Using this new method the analysis produced a slight shift to earlier retention times on the Alliance HPLC System, with no changes in selectivity (Figure 3). The difference in retention times across the two instruments decreased and all of the compounds were within 2.5% difference as compared to the Agilent 1100 Series LC System (Table 3). Furthermore, for the complex separation, similar resolution was obtained for the critical pair (Peaks 3 and 4).

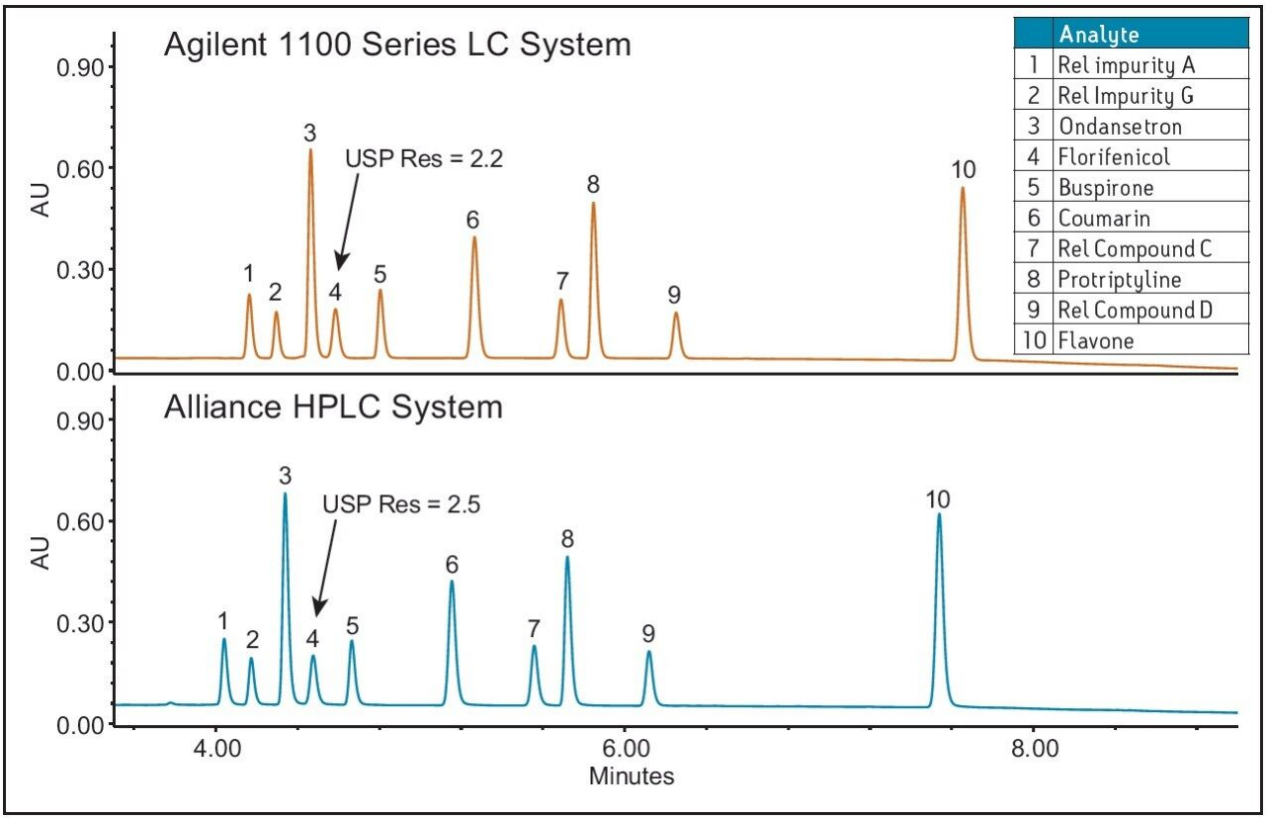

Figure 3. Separation of complex mixture, including ondansetron and related compounds, on both an Agilent 1100 LC Series System and an Alliance HPLC System. The gradient method on the Alliance HPLC System was adjusted to compensate for the dwell volume difference as compared to the Agilent 1100 Series LC System. Similar retention were observed for all compounds and the USP Resolution of the critical pair was preserved.

Compound	Agilent 1100 Series LC System	Alliance HPLC System with gradient delay compensation	% Deviation
Rel impurity A	4.17	4.07	2.42
Rel Impurity G	4.30	4.20	2.28
Ondansetron	4.47	4.37	2.17
Florifenicol	4.59	4.50	1.87
Buspirone	4.81	4.70	2.23
Coumarin	5.27	5.19	1.54
Rel Compound C	5.69	5.60	1.65
Protriptyline	5.85	5.76	1.44
Rel Compound D	6.25	6.16	1.42
Flavone	7.66	7.60	0.74

Table 3. Retention times observed on the Agilent 1100 Series LC System and the Alliance HPLC System with adjustments to compensate for dwell volume differences between the two systems. The retention times were within 0.11 minutes or less than 2.5% difference on the Alliance HPLC System as compared to the Agilent 1100 Quaternary Series LC System. All values are average of six replicate injections.

Conclusion

When transferring a method across two different HPLC instruments, it is typically preferred to have no changes or adjustments in the method. While methods transfer across the same instrument model may be accomplished with minimum intervention, challenges can be encountered when switching between instrument manufacturers. In this example, a challenging separation was transferred from one vendor's HPLC system to another, specifically from an Agilent 1100 Series LC System to an e2695 Alliance HPLC System.

The initial method transfer was performed without adjustments to the method for the gradient dwell volume. The results produced retention time deviations within 5% and relative retention within 0.13 minutes. However, by compensating for the differences in the gradient delay volume the retention time differences decreased to less than 0.11 minutes of 2.5% difference, with no loss of resolution for the critical pair. In this example, a challenging separation was successfully transferred from an Agilent 1100 Series LC System to an

HPLC System.

References

1. Easy Transfer of Standard HPLC Methods to the Agilent 1200 Series Rapid Resolution System. Agilent Technologies, Inc; 2006.
2. Chapter <621> CHROMATOGRAPHY United States Pharmacopeia and National Formulary (USP 37-NF 32 S1) Baltimore, MD: United Book Press, Inc.; 2014. p. 6383.
3. Protocol for Gradient Delay (Dwell Volume) Measurement. Transferring Compendial HPLC Methods to UPLC Technology. Application Notebook: Waters Corporation; 2013. p. 67–8.

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