

## Method Transfer of a Fast Gradient from a Binary HPLC to an Arc HPLC System

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

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## Abstract

This application brief demonstrates the method transfer for a fast gradient method from an HPLC legacy system to the Arc HPLC System. The quaternary Arc HPLC System provides greater injection precision with retention time repeatability comparable to the binary HPLC systems.

### Benefits

- Method transfer of a fast gradient from a binary HPLC to quaternary Arc HPLC System
- High injection precision for HPLC analyses with the Arc HPLC System

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## Introduction

With the globalization of resources, method transfer across different laboratories and geographical locations has been an area of increased interest. While many scientists prefer to transfer methods across similar systems, this is not always possible. While the different pumps, quaternary vs. binary, can impact retention time characteristics, other aspects, including injection precision, are a characteristic of the sample manager. Regardless of the system, both injection and retention time precision are important performance characteristics of any method. In this example, we have demonstrated the transferability and effect on key assay performance characteristics of a fast gradient method from a legacy binary HPLC system to an Arc HPLC System.

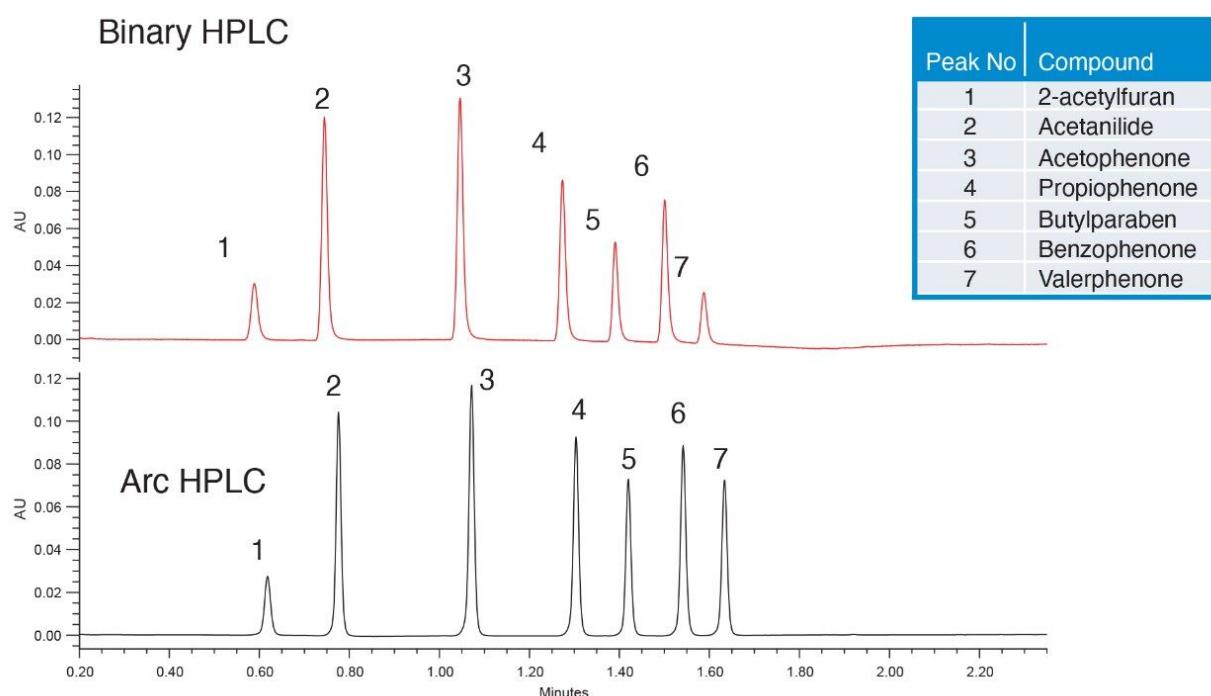
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## Results and Discussion

Method transfer across binary and quaternary systems present unique challenges because of differences in gradient delay or dwell volumes, gradient delivery mechanism, and mixing across the two types of solvent managers (high pressure vs. low pressure). While some methods are better suited for one type of pump over the other,<sup>1</sup> many routine HPLC methods will meet system suitability criteria on either system. Specifically, these are methods that are within the performance specifications of both types of pumps, including starting %A and B, and flow rates. Under these conditions, methods are typically transferable and may only require

adjustments for delay volume if absolute retention time criteria are used.

In this example, a very fast gradient separation of a small molecule mixture was transferred from a legacy binary HPLC system to the Waters Arc HPLC System. Methods with this very rapid gradient are susceptible to changes in mixing modes and gradient delay volume. Comparison of the separation shows a shift in retention time between the two systems (Figure 1 and Table 1). This shift corresponds to the difference in the gradient delay volumes of these two specific systems, or roughly 0.200 mL. While absolute retention time may be a critical aspect of a separation, relative retention times are commonly used to reduce the impact of the delay volume on peak confirmation or identification. In this case, comparison of the relative retention time, using valerophenone as a reference, demonstrates the same selectivity across the two methods.



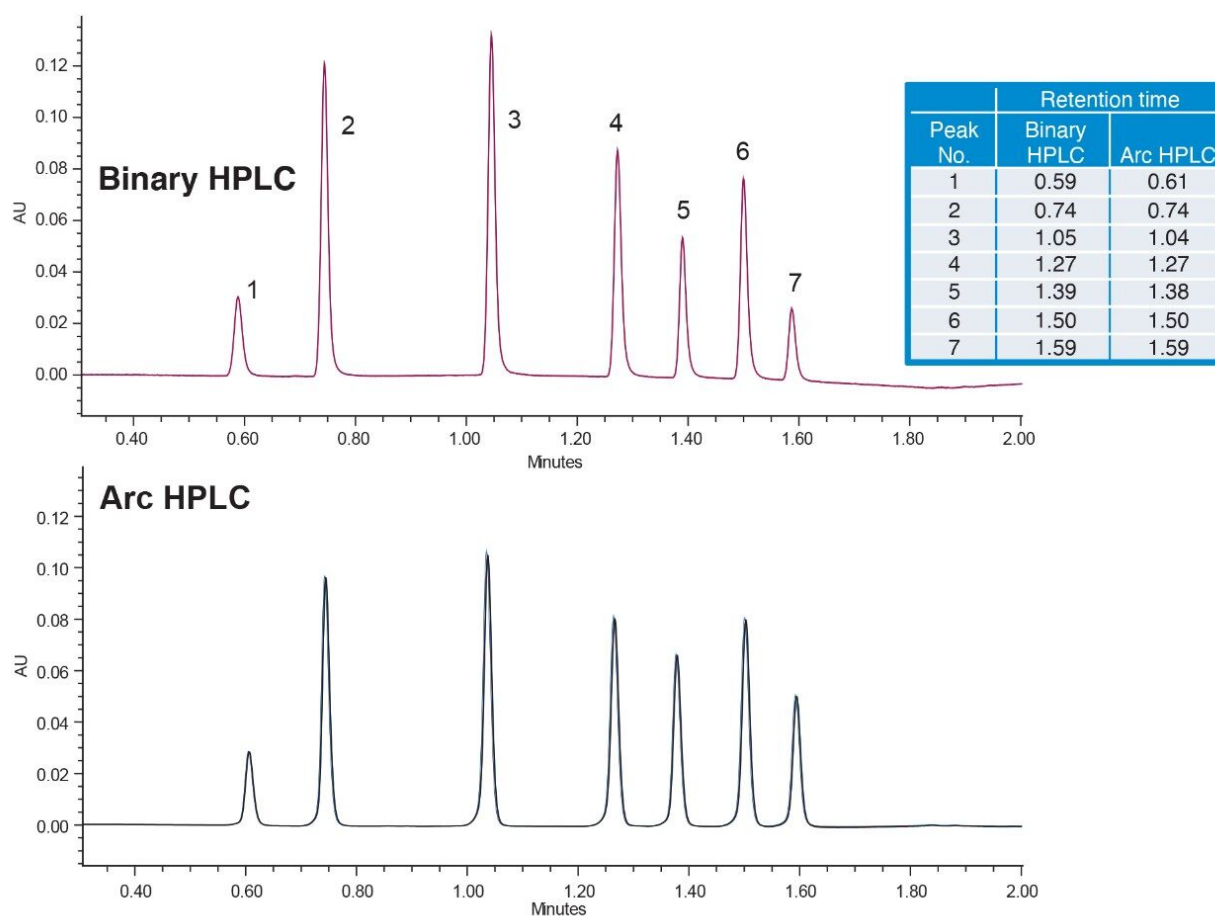
*Figure 1. Method transfer of a small molecule separation using a fast gradient from a binary HPLC system to the Arc HPLC System. Gradient conditions were 10-80% B in 1.5 min, with A: water, B: acetonitrile. Waters standard was diluted in 90/10 water/acetonitrile with a 20- $\mu$ L injection. Separation fidelity was preserved, including relative retention times and USP resolution. Relative peak area varies due to sample stability.*

Compound	Retention time		RRT		USP resolution	
	Arc HPLC	Agilent 1110	Arc HPLC	Agilent 1100	Arc HPLC	Agilent 1100
2-acetylfuran	0.642	0.589	0.38	0.37	n/a	n/a
Acetanilide	0.80	0.74	0.48	0.47	5.91	6.00
Acetophenone	1.11	1.05	0.66	0.66	11.58	13.00
Propiophenone	1.34	1.27	0.80	0.80	8.80	9.50
Butylparaben	1.45	1.39	0.87	0.88	4.33	5.10
Benzophenone	1.58	1.50	0.94	0.95	4.70	4.80
Valerphenone	1.67	1.59	1.00	1.00	3.45	3.70

*Table 1. Retention time and USP resolution comparison of a fast gradient separation on a binary HPLC and Arc HPLC System.*

Further confirmation of the separation fidelity is provided by other chromatographic properties, including USP resolution of the two separations (Figure 1 and Table 1). While there was slight variability of some USP resolution values, all the peaks were well separated with USP resolution >2.0. Both systems also produced USP tailing of 1.0-1.3, within accepted values. Slight system differences (e.g., fittings and preheaters) could potentially contribute to some variability. In addition, the two analyses were performed at different time points, with two different, specific columns and sample preparations. As a result, column-to-column variability cannot be ruled out as slightly impacting basic chromatographic performance characteristics.

Given the differences across systems in the gradient delay volume, adjustments for gradient delay are generally permitted in method transfer.<sup>2</sup> To ensure the same separation is obtained on both systems, Gradient SmartStart was used to adjust the gradient start relative to the injection without modifications to the gradient table.<sup>3</sup> Based on the results, adjustments were made for the gradient to start 0.05 min before injection. This resulted in lower delay volume on the Arc HPLC System and absolute retention times more closely matching the binary HPLC system, eliminating the need for relative retention times (RRT) requirements (Figure 2).



*Figure 2. Overlay of six replicate injections of a method transfer from a binary HPLC System to the Arc HPLC System with delay volume adjustments. Using Gradient SmartStart, the gradient delay on the Arc HPLC System was adjusted for comparable retention times to the binary HPLC. Six replicate injections were performed to assess retention time and area precision. Relative peak area varies due to sample stability.*

To assess the precision of both the pump and the sample manager, six replicate injections were performed on each system. As described earlier, retention time precision is a function of the mobile phase delivery system, while area or injection precision is impacted by performance of the sample manager. Comparing the two systems, we can see low retention time standard deviations for each system (<0.002 min or 7  $\mu$ L), well within typical specifications for such a fast method, indicating reproducible mobile phase delivery, regardless of pump type. The peak area %RSDs demonstrate the high precision of the Arc HPLC System sample manager at injection volume of 20  $\mu$ L with values of <0.2% (Figure 3).

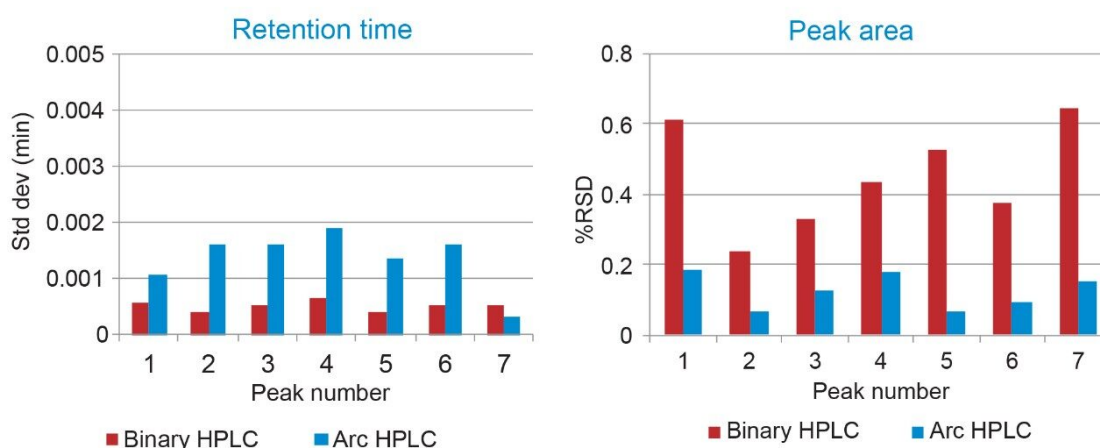


Figure 3. Retention time standard deviation and injection precision comparison of the binary HPLC and Arc HPLC systems for fast gradient. Six replicate injections were used for results.

## Conclusion

For many typical HPLC methods, transfer from a binary system to a quaternary can produce comparable results. Specifically, the transfer of a challenging, very fast gradient separation on a 4.6 x 50 mm column demonstrated similar retention time and resolution results. Small differences in absolute retention time were observed due to gradient delay of each pump, requiring an evaluation of the relative retention times. By adjusting the gradient start relative to the injection, with Gradient SmartStart, comparable absolute retention times were observed on both systems. High retention time precision was observed on both pumps with standard deviations of less than one second or 7  $\mu$ L, while the injection precision on the Arc HPLC System was superior to the legacy binary LC system.

## References

1. Simeone, J.; Hong, P.; and McConville, P.R. Performance Comparison of Binary Ultra High-Performance Liquid Chromatography Systems from Multiple Vendors for Methods which Utilize Long, Shallow Gradients. Waters Application Note, [720006369EN](#) <

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2. <621> CHROMATOGRAPHY. In United States Pharmacopeia and National Formulary (USP 39 NF34), United Book Press, Inc.: Baltimore, MD, 2016, 459–471.
3. Hong, P. and McConville, P.R. Using Gradient SmartStart Technology and an ACQUITY UPLC H-Class System to Emulate an Agilent 1100 Series LC System Separation for Impurity Testing. Waters Application Note, [720005332EN](#) <<https://www.waters.com/waters/library.htm?cid=511436&lid=134851594>> , 2015.

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