Waters
THE SCIENCE OF WHAT'S POSSIBLE.®

Simplifying Methods Transfer: Novel Tools for Replicating Your Established Methods on an ACQUITY Arc System

Paula Hong, Richard Andrews, Peyton C. Beals, and Patricia R. McConville
Waters Corporation, Milford, MA, USA

APPLICATION BENEFITS

- Ability to select between two flow paths with different dwell volumes using Arc Multi-flow path™ technology
- Ability to adjust gradient start relative to injection with a single setting using Gradient SmartStart
- Adjustment of gradient start using units of time rather than volume for intuitive methods transfer adjustment

WATERS SOLUTIONS

Gradient SmartStart

ACQUITY® Arc System with Arc Multi-flow path technology

Empower 3 FR2

KEY WORDS

Dwell volume, Methods transfer, Gradient SmartStart, ACQUITY Arc system

INTRODUCTION

Retention shifts in methods transfer are commonly observed. Retention can be impacted by differences in system and pump characteristics such as dwell volume and mixing characteristics. Many laboratories require there be no adjustments to a method, particularly for validated methods. If adjustments are necessary, consideration is generally made to be in accordance with regulatory guidelines. With reference to dwell volume, chapter <621> of the USP monograph states "If adjustments are necessary, change in... the duration of an initial isocratic hold (when prescribed), and/or dwell volume adjustments are allowed."[1] While adjustments for dwell volume can be manually entered into a gradient table, this approach requires calculations and manual changes to the gradient table; both of which require additional time and effort. Using a feature in the software to adjust the time between the start of the gradient and the point of injection, the duration of the gradient hold can be adjusted. This is accomplished without the need to make changes to the gradient table allowing systems – such as the ACQUITY Arc™ System – to mimic systems with different dwell volumes. By being able to enter the value directly in time, methods transfer can be streamlined by measuring the difference in retention time and adjusting the initial hold accordingly.

EXPERIMENTAL

Sample description

In a scintillation vial, 20 µL of Preparative Chromatography Mix Standard (p/n 186006703) and 100 µL of ACQUITY UPLC MS Start-Up Solution 2 (p/n 700002741) were combined with 880 µL of 30:70 H_2O: acetontrile. The final concentration of each component is listed below.

Peak no.	Analyte	Concentration
1	Acetaminophen	0.01 mg/mL
2	Caffeine	0.01 mg/mL
3	Diphenhydramine	0.1 mg/mL
4	Reserpine	0.01 mg/mL
5	Sulfadimethoxine	0.01 mg/mL
6	Flavone	0.1 mg/mL
7	Diclofenac	0.1 mg/mL

Method conditions

LC conditions

LC system:	ACQUITY Arc System, Path 1
Column heater:	CH30-A with active preheating
Detection:	2998 PDA with low dispersion analytical flow cell
Column temp.:	30 °C
Column:	XSelect® CSH C_{18}, 4.6 x 250 mm, 5 µm (p/n 186005291)
Injection volume:	10 µL
Flow rate:	2.0 mL/min
Mobile phase A:	0.1% (v/v) Formic acid in water
Mobile phase B:	0.1% (v/v) Formic acid in acetonitrile
Wash solvent:	50/50 Water/acetonitrile
Purge solvent:	90/10 Water/methanol
Seal sash:	90/10 Water/methanol
Wavelength:	260 nm
Sampling rate:	5 Hz
Time constant:	Normal
Gradient:	15 to 35% B in 3 min, 35 to 95% B in 2 min
Agilent system:	Agilent 1260 Bio Quaternary LC System (Quat Pump: G5611A; HiP ALS: G5667A; Column Compartment: G1316C; DAD,VL+:G1315C)

Data management

Chromatography software:	Empower 3 FR2 SR2

RESULTS AND DISCUSSION

A sample containing two major components and five minor components was analyzed on an Agilent 1260 Quaternary LC System. The method was transferred from the Agilent 1260 System to the ACQUITY Arc System with no changes in method conditions or column. Since retention time shifts for gradient separations can, in part, be attributed to the dwell volume differences of the instruments, the ACQUITY Arc System was configured using Flow Path 1 of the Arc Multi-flow path technology. This instrument feature uses of a six-port valve to enable switching of the flow from the pump to the injector between two different paths, each with a different physical volume (Figure 1). Selection of Path 1 results in a dwell volume of approximately 1.1 mL on the ACQUITY Arc System – a value that is typical of many HPLC systems. Path 2, which was not selected for this study, results in a system dwell volume of 0.76 mL.

Using Flow Path 1, the ACQUITY Arc System produced a separation with similar retention times to that of the Agilent 1260 Quaternary system (Figure 2, Table 1). The retention times for all the analytes were within 3% of the initial separation observed on the Agilent 1260 Infinity System. A noticeable shift, however, was observed for peak 3, which had a retention time difference of 0.10 min. This represented a shift 5–10 times greater than observed for all the other peaks. Since this peak was shifted more than the peaks that eluted earlier or later, the difference could not be entirely explained by dwell volume. Inspection of the programmed gradient revealed the peak eluted in the middle part of the first step of the gradient. While there are many factors that can impact a separation, differences in pump characteristics, such as the gradient delay, mixing and shape of the gradient can impact retention. Additional studies, not described here, indicate the shift in retention of peak 3 is influenced by gradient shape.

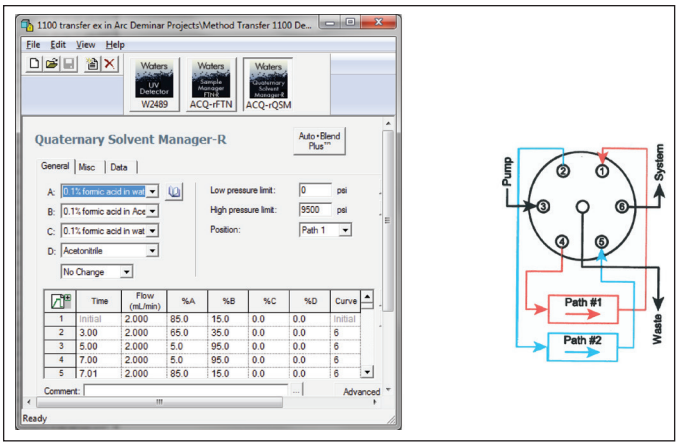

Figure 1. Instrument editor and schematic illustrating selection and configuration of Multi-flow path technology on the ACQUITY Arc QSM-R System. For methods transfer from an Agilent 1260 System, Path 1 (red) was selected in the instrument editor for a system dwell volume of approximately 1.1 mL.

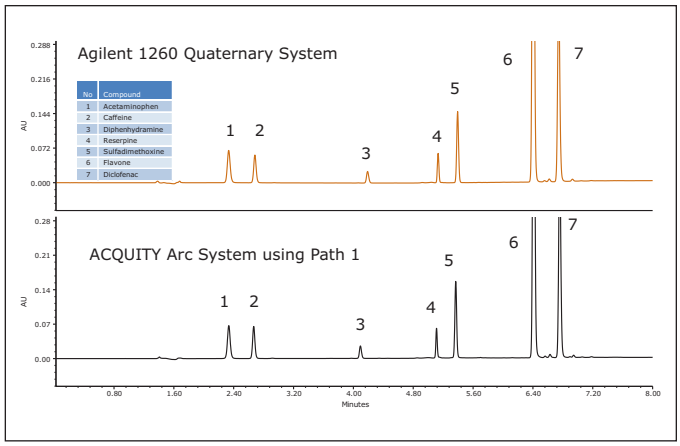

Figure 2. Overlay of a mixture of standards on the Agilent 1260 Quaternary LC System (top chromatogram) and the ACQUITY Arc System (bottom chromatogram). Similar retention times were observed on the Agilent and the ACQUITY Arc System using Path 1.

No.	Compound	Agilent 1260 Infinity Quaternary System	ACQUITY Arc System	Retention time shift	% Deviation
1	Acetaminophen	2.33	2.33	0.00	-0.04
2	Caffeine	2.68	2.67	-0.01	-0.37
3	Diphenhydramine	4.19	4.09	-0.10	-2.39
4	Reserpine	5.13	5.11	-0.02	-0.39
5	Sulfadimethoxine	5.39	5.37	-0.02	-0.37
6	Flavone	6.40	6.41	0.01	0.16
7	Diclofenac	6.74	6.76	0.02	0.30

Table 1. Comparison of retention times on an Agilent 1260 Quaternary Series LC System and an ACQUITY Arc System using Path 1. All retention times were within 0.10 minutes. The largest shift in retention time was observed for peak 3.

One approach to adjust retention includes adjusting the initial hold of the separation. On the ACQUITY Arc System, the gradient can be adjusted to occur "at injection", "after injection" or "before injection", thereby allowing the system to emulate other chromatographic systems with larger or smaller dwell volumes (Figure 3). This dwell volume adjustment (Gradient SmartStart), which does not require any changes to the gradient table, can be entered in either time or volume, allowing for the flexibility required to emulate different HPLC systems.

We can use this feature for the methods transfer example illustrated earlier. While the method transfer results produced retention times within 5% of the original separation – which represents a typical retention time window for peak identification – more stringent retention time criteria can be met by adjustment of the dwell volume. An overlay of the analyses on the Agilent 1260 and the ACQUITY Arc Systems illustrate the noticeable shift in retention time, particularly for peak 3. Measuring the retention time of each peak we can determine the shift for peak 3 is 0.1 min, while the remaining peaks have a much smaller shift of 0.01–0.02 minutes (Figure 4). Using the aforementioned approach, the gradient start was adjusted to begin 0.05 min after injection on the ACQUITY Arc System to improve the retention time correlation for peak 3. This value was selected since it is the mid-point of the retention time shifts observed.

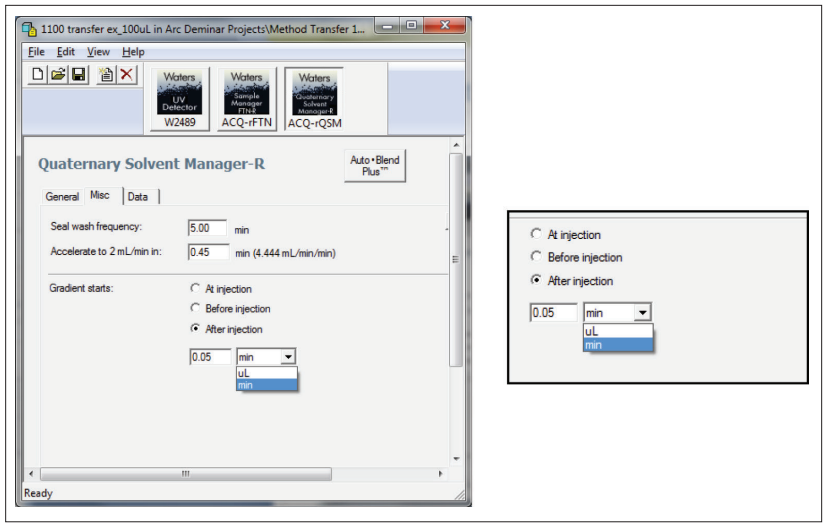

Figure 3. Instrument editor showing Gradient SmartStart feature. Gradient start can be adjusted to occur at, before, or after injection without any changes to the gradient table. The adjustment can be made in µLs or minutes. This feature allows the initial hold of the separation to be adjusted to mimic the dwell volume of another system. For the example in this application note the gradient start was adjusted 0.05 min after injection.

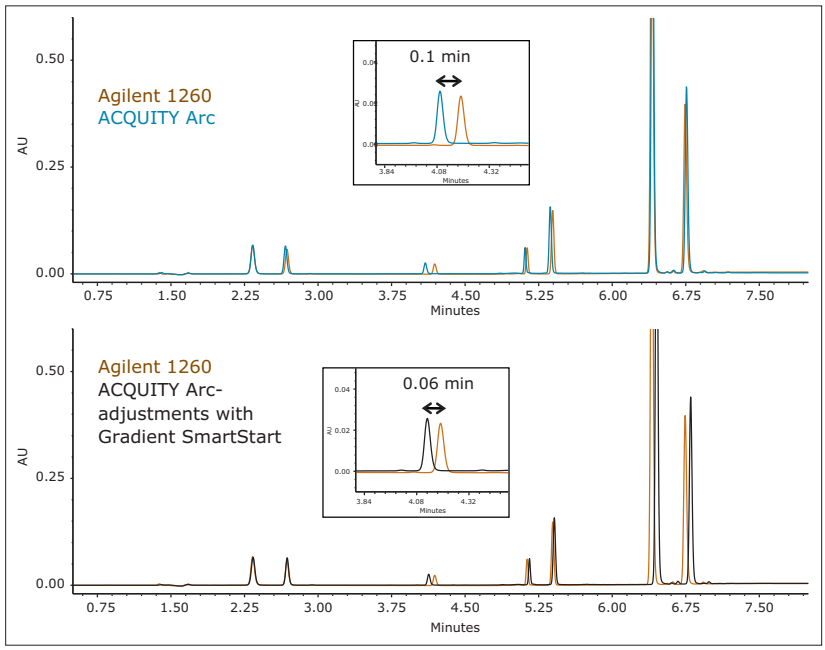

Figure 4. Fine-tuning methods transfer using Gradient SmartStart. Overlay of a mixture of standards on the Agilent 1260 Quaternary LC System and the ACQUITY Arc System (top chromatogram). In this method transfer example, a greater retention time shift is observed for peak 3 (inset) than for the other analytes. To reduce the retention time difference, the method was re-run on the ACQUITY Arc System using Gradient SmartStart to begin the gradient 0.05 min after injection, thereby increasing the initial hold. Overlay of this injection with that from the Agilent 1260 System (bottom chromatogram) shows the retention shift for peak 3 (inset) is reduced to 0.06 min while the other analytes are still within 0.05 min.

The effect of adjusting the initial hold of the method produced retention time shifts of between 0.01 to 0.05 minutes for the analytes (Table 2). The change in retention times of the first two peaks (acetaminophen and caffeine) was minimal (< 0.02 minutes), while the later eluting peaks had retention time shifts much closer to the gradient adjustment of 0.05 min. For the analyte of interest (Peak 3) the retention times shifted 0.06 min earlier, resulting in a retention time deviation of 1.5% relative to the Agilent 1260 Infinity System (Figure 5). The effect of the adjustment on retention may vary depending on the mobile phase composition at elution. When analytes elute in an isocratic step or under constant mobile phase conditions, retention times will be shifted similar to the change in gradient start. When peaks elute under gradient conditions, changing the timing of the solvent delivery can have a more complex effect on retention.

Nevertheless, the adjustment of the gradient start enabled fine-tuning of the retention. The change of the gradient start resulted in a maximum retention time deviation of 1.4% compared to the Agilent 1260 separation. As compared to the first analysis on the ACQUITY Arc System, adjustment of the gradient start produced similar average deviations with lower maximum deviation or difference.

No.	Compound	Agilent 1260 Infinity Quaternary System	ACQUITY Arc-Adjustment with Gradient SmartStart	Retention time shift	% Deviation
1	Acetaminophen	2.33	2.34	0.01	0.43
2	Caffeine	2.68	2.69	0.01	0.37
3	Diphenhydramine	4.19	4.13	-0.06	-1.43
4	Reserpine	5.13	5.16	0.03	0.58
5	Sulfadimethoxine	5.39	5.41	0.02	0.37
6	Flavone	6.40	6.45	0.05	0.78
7	Diclofenac	6.74	6.80	0.06	0.89

Table 2. Comparison of retention times on an Agilent 1260 Quaternary LC System and an ACQUITY Arc System using Gradient SmartStart to adjust the gradient start 0.05 min after the injection. All retention times were within 0.06 minutes. The retention time shift for peak 3 decreased from 0.10 min to 0.06 minutes.

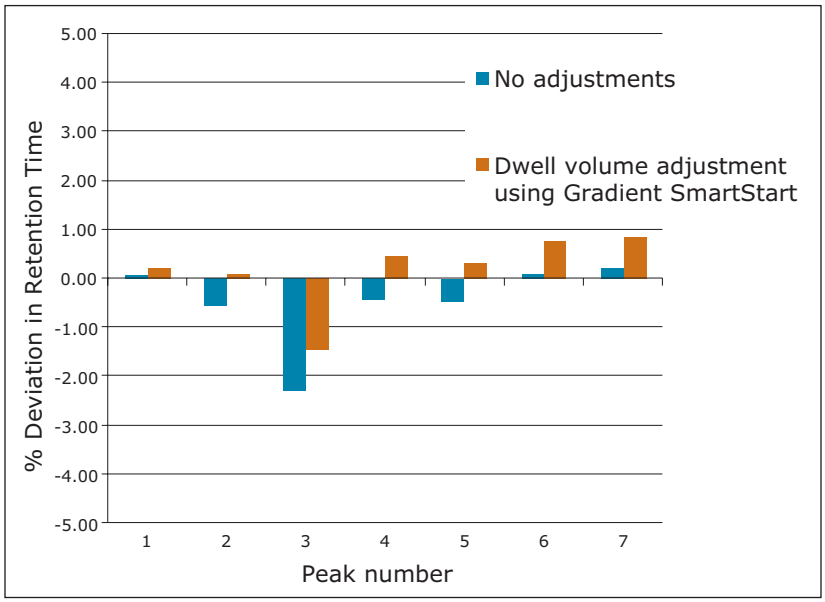

Figure 5. Fine-tuning methods transfer using Gradient SmartStart. For methods transfer from an Agilent 1260 Quaternary LC System to an ACQUITY Arc System all the retention times were within 5% of the original method (blue columns). Using Gradient SmartStart to adjust the dwell volume of the system, the resulting transfer produced deviations of less than 2% for all compounds, an improvement over the original transfer.

CONCLUSIONS

One of the prime capabilities of the ACQUITY Arc System is the ability to easily transfer traditional/legacy HPLC methods (e.g. USP, or NF) from other HPLC systems. In this example, a method transfer from another manufacturer's HPLC System to an ACQUITY Arc system was performed using the Path 1 of the Arc Multi-flow path technology. This flow path, which was designed for methods transfer, allows the ACQUITY Arc System to mimic the dwell volume of a typical HPLC system. The results produced retention times within 5% of the original method. Additional fine-tuning of retention times was accomplished using Gradient SmartStart Technology, which adjusts the initial hold of the method without requiring any changes to the gradient table. The combination of these two features enables methods transfer to an ACQUITY Arc System in just two injections for a simplified methods transfer approach, including the following:

1. Transfer method to an ACQUITY Arc using appropriate Flow Path (1 or 2).
2. Compare chromatograms and evaluate retention.
3. If retention does not meet method criteria, adjust gradient start using Gradient SmartStart. Re-run method.

References

In *United States Pharmacopeia and National Formulary* (USP 37-NF 32 S2).; United Book Press, Inc.: Baltimore, MD, 2014; Vol., p 6376.

Waters

THE SCIENCE OF WHAT'S POSSIBLE.®

Waters Corporation
34 Maple Street
Milford, MA 01757 U.S.A.
T: 1 508 478 2000
F: 1 508 872 1990
www.waters.com