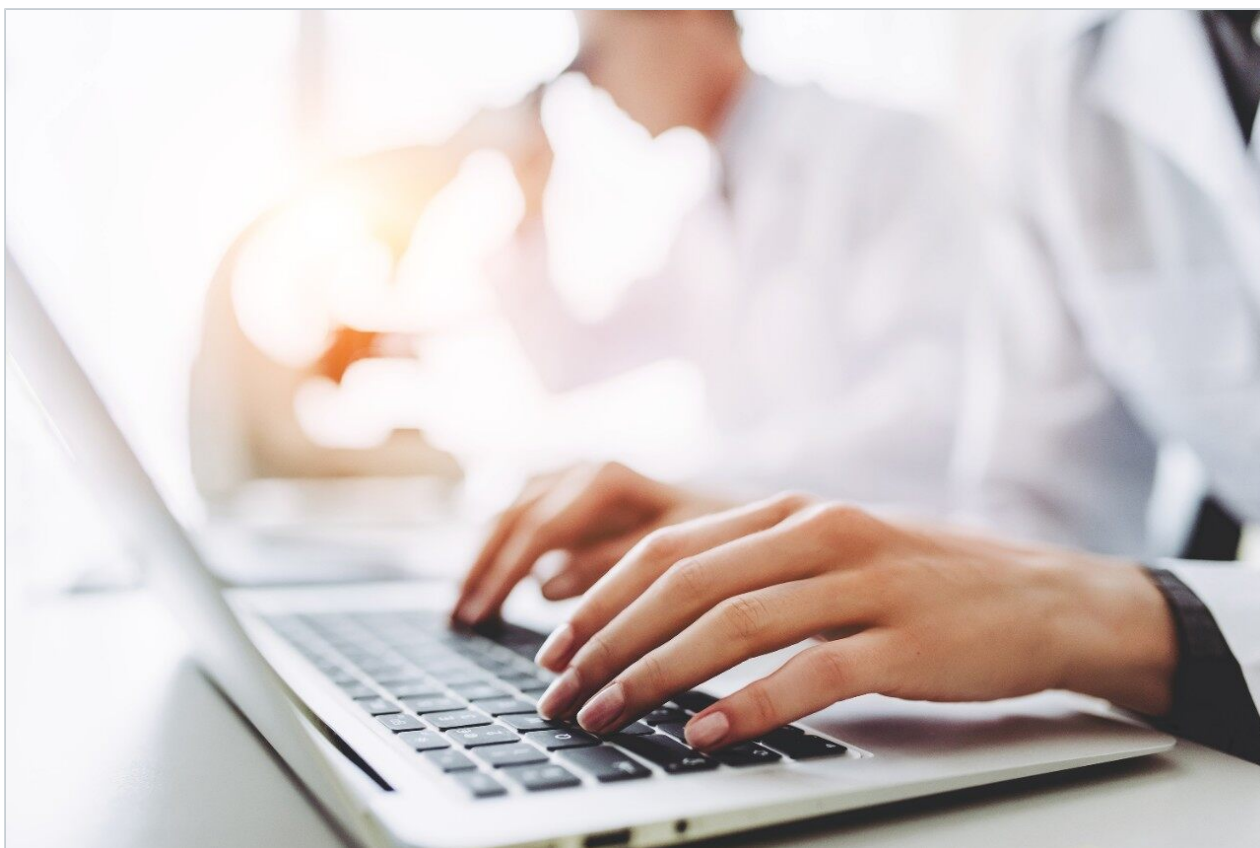


Application Note

## AMDS Method Transfer Series Instrument-To-Instrument Dwell Volume Differences

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Waters Corporation



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

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### Abstract

This application brief demonstrates about Waters Automated Method Development System (AMDS) which provides tools to effortlessly transfer a method between instruments.

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## Introduction

Traditional manual method development and transfer can be a labor-intensive, time-consuming, and often imprecise process. The process becomes even slower and more frustrating when the method that needs to be transferred requires modification in the end user's lab. The Waters Automated Method Development System (AMDS) is a tool that can increase efficiency and throughput to streamline these issues.

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## Results and Discussion

### Dwell volumes' importance in instrument upgrades

As technology advances, more and more labs are upgrading equipment. Unfortunately, when an instrument is upgraded, quite often the method performs differently than it did on the older system. Retention times don't match, and sometimes the chromatographic separation or resolution suffers.

The first step in successfully transferring a method is to gather information about the new and existing system. One critical piece of information is obtained by measuring dwell volume, also known as the gradient delay volume (See Figure 1). Dwell volumes are often described as small values to promote the quality of the system for marketing purposes.

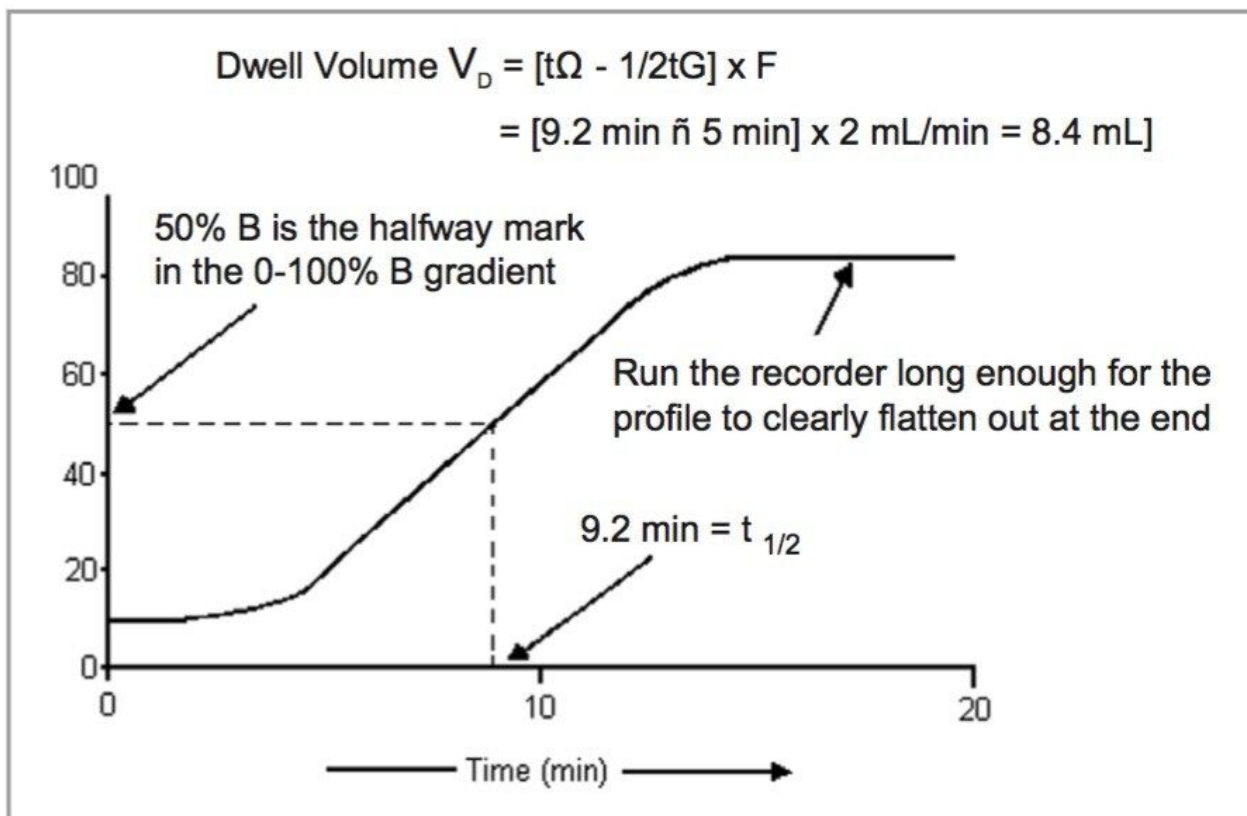


Figure 1. Determining dwell volume.

However, many times vendors are not actually showing the chromatographic sense of dwell volumes, the actual point of homogenous gradient mixing and lift-off point of a gradient curve. These volumes are generally greater.

Older HPLC systems generally have greater dwell volumes than today's modern technology and can range from 2-6 mL. In newer instruments, the volume range can be 0.5-1.3 mL and less.

1. Remove the column from the system and connect the injector to the detector by means of a short piece of 0.010 in. i.d. tubing.
2. For solvent A, use HPLC-grade water; for solvent B, add about 0.1% acetone to water (methanol or acetonitrile can be used instead of water).
3. Set the detector wavelength to the absorbance maximum of the probe (265 nm for acetone).
4. Program a 0-100% B linear gradient in 10 min at 2 mL/min (the exact conditions are not critical; just make sure the gradient volume is at least 20 mL) with a hold at 100% B.

5. Determine the dwell time by first locating the time at the midpoint of the gradient ( $t_{1/2}$ ) (half the vertical distance between the initial and final isocratic segments; 9.2 for the figure above). Then, subtract half the gradient time ( $10 \text{ min}/2 = 5 \text{ min}$  for the present example) from  $t_{1/2}$ . The result is the dwell time ( $t_D$ ) [ $9.2 \text{ min} - 5 \text{ min} = 4.2 \text{ min}$ ]. Convert the dwell time ( $t_D$ ) to the dwell volume ( $V_D$ ) by multiplying by the flow rate (F) [ $4.2 \text{ min} \times 2 \text{ mL/min} = 8.4 \text{ mL}$ ].

### Method transfer can be as easy as 1-2-3

Method transfer can be as easy as 1-2-3 Using the method transfer capabilities of the Waters AMDS, nearly identical results can be obtained from different HPLC dwell volumes for a seamless transfer between instruments.

In Figure 2, the original method was developed on a system with a dwell volume equal to 2.22 mL. The Waters Alliance System to which it was transferred measured a dwell volume equal to 1.05 mL. Once the automated calibration experiments were performed and automatically entered into Drylab 2000plus, a chromatographic match was achieved by manipulating the Drylab Gradient Editor.

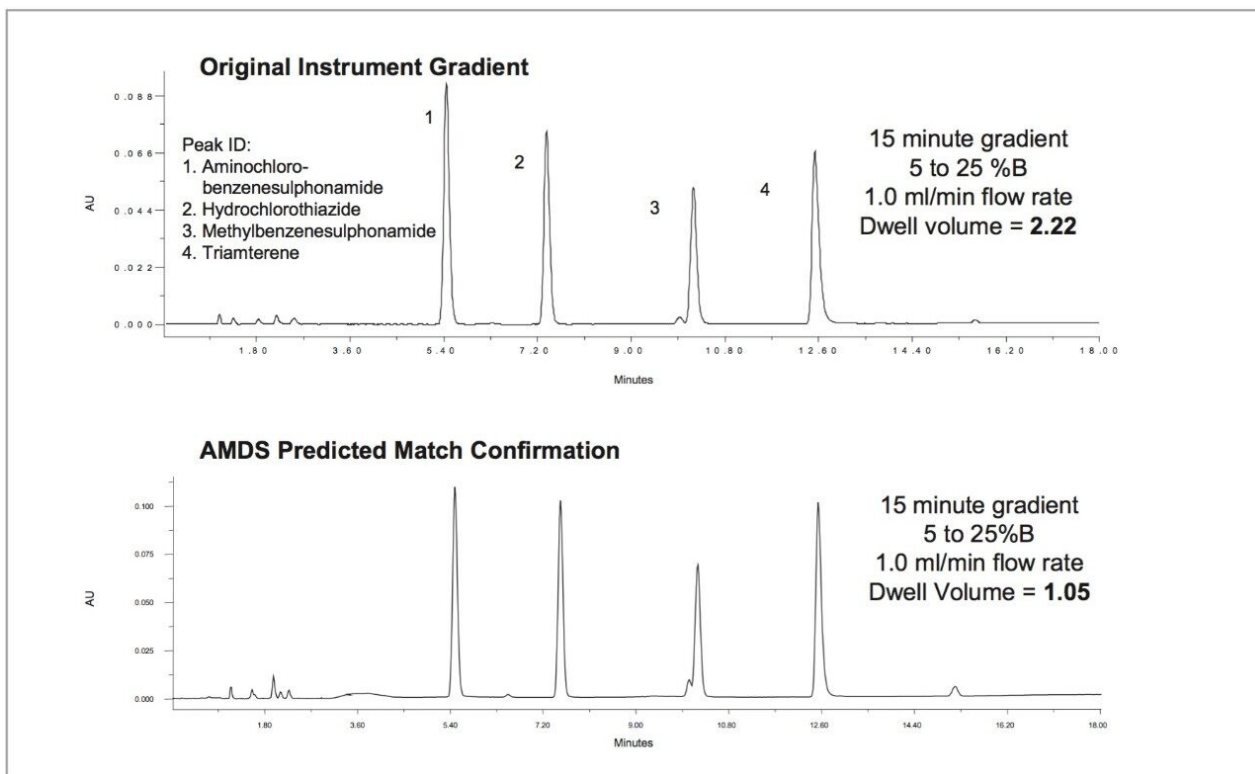


Figure 2. Match Confirmation.

The simulated chromatogram was “de-optimized” to show compatibility with an older system’s method. Drylab predicted an isocratic hold would achieve the matching conditions on the Alliance System.  
 \*Note: Adjustments to the gradient within the simulation could also help the chemist identify mixing inconsistencies between systems.

The following simple steps can help you transfer any method when using the original chromatogram as a reference:

1. Perform AMDS on the sample and select “existing separation” where it asks for which kind of separation;
2. Choose “Allow user intervention at the end of each sample set” on the last wizard screen;
3. Review the results entered in Drylab and make adjustments in the Resolution Plot, Gradient Editor, or both, such that the simulation matches the reference chromatogram. Run the conditions to verify accuracy.

In this example, a gradient hold at the beginning of the injection was sufficient enough to yield a matching chromatogram. In some cases, however, some further manipulations with the Resolution Plot may be needed to match chromatograms between systems with varying gradient proportioning valves and mixing performance.

## Extremely accurate predictions

AMDS also accurately predicts retention times, resolution, solvent used, average  $k'$  for any changes in column dimensions, solvent strength, temperature, or gradient modifications. Comparing actual to predicted results generally yields differences that can range from 0 to 0.8 minutes depending on the accuracy of the measured dwell volume, extracolumn volume, and integrated data (Figure 3).

Peak	Name	Original RT	AMDS predicted RT	AMDS actual RT
1	Aminochloro-benzenesulphonamide	5.448	5.48	5.491
2	Hydrochlorothiazide	7.374	7.49	7.538
3	Methylbenzene-sulphonamide	10.196	10.19	10.198
4	Triamterene	12.531	12.53	12.537

Figure 3. Drylab Prediction Accuracy.

This shows the accuracy of predicted retention times (RT) compared to actual results when verified by the AMDS.

## Obtain better methods

With the Waters Automated Method Development System, you can develop more robust and less problematic methods (See Figure 4). AMDS can perform an optimal LC chromatogram without the need for additional experiments. By selecting "continue analysis until complete" on the last setup wizard screen, you can optimize your method according to goals that you suggest. The end result not only provides the you with Drylab2000 simulation data, but also a method that may result in a faster, more robust, chromatogram.

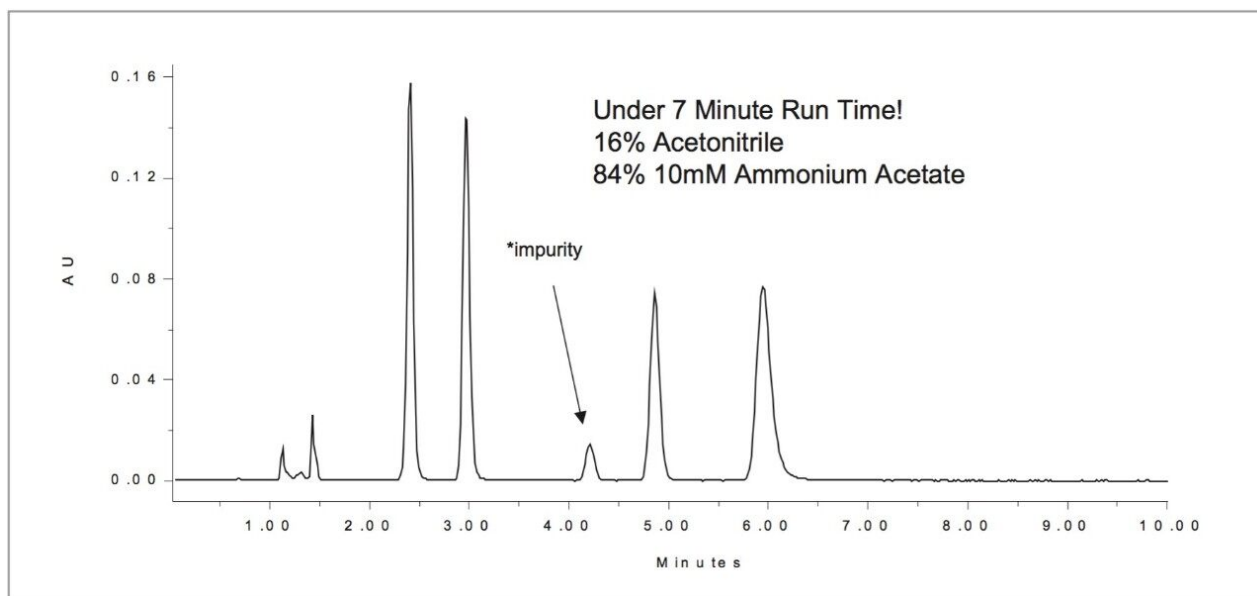


Figure 4. Optimizing your new method.

Based on the same separation, AMDS optimized a more robust isocratic and shorter run time solution. Issues were also solved with a problematic degradant peak that was not completely resolved in the original method.

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## Conclusion

In many cases, when upgrading to new lab equipment, methods are developed in haste due to time constraints. The Waters AMDS provides the tools necessary to effortlessly transfer a method between instruments.

Moving to newer technology is made easier once accurate dwell volume measurements are obtained. In a three-step process, you can obtain valuable chromatographic information — displayed colorfully in a Drylab resolution plot — that simulates changes using different variables, as well as providing indications of method robustness.

The Gradient Editor is likewise an excellent tool that can simulate mixing differences between instruments, simulating multiple gradients or gradient holds. Prediction accuracies are usually within  $\pm 1$  minute and your end result is often a more optimized method.

The Waters AMDS is an excellent tool to transfer methods efficiently while ultimately saving time and money.

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