

Seamless Method Transfer between UPLC System Platforms

GOAL

Separation of a series of barbiturates is used to demonstrate new software functionality which easily compensates for system volume differences that impact direct transferability between UPLC® platforms.

BACKGROUND

Validated methods are often transferred between laboratories and sites, requiring data quality to be maintained in this transfer. In such cases, the method development lab and the receiving implementation lab may employ different chromatography systems that have different system volumes. System volume differences do not impact the transfer process of an isocratic separation. In gradient separations, however, the presence of an initial isocratic hold due to differences in system volume requires recalculation of chromatographic parameters. In the past, it was easy to transfer methods from systems with large system volumes to systems with smaller system volumes but not the reverse, as one could not account for negative system volumes. Herein, we describe the use of a new software tool to aid the process of transferring methods between systems regardless of their system volumes.

New software functionality streamlines the transfer of methods between the ACQUITY UPLC H-Class System and the ACQUITY UPLC I-Class System.

THE SOLUTION

Both the ACQUITY UPLC® H-Class and the ACQUITY UPLC I-Class systems deliver true UPLC performance, resulting in high resolution separations. The ACQUITY UPLC H-Class System was designed to provide ultimate flexibility for UPLC method development and routine analysis. The ACQUITY UPLC I-Class System was designed and optimized to offer the lowest extra-column band spread and deliver ultimate separation efficiency. System volume differences must be accounted for when transferring gradient methods between these systems to achieve the same separation.

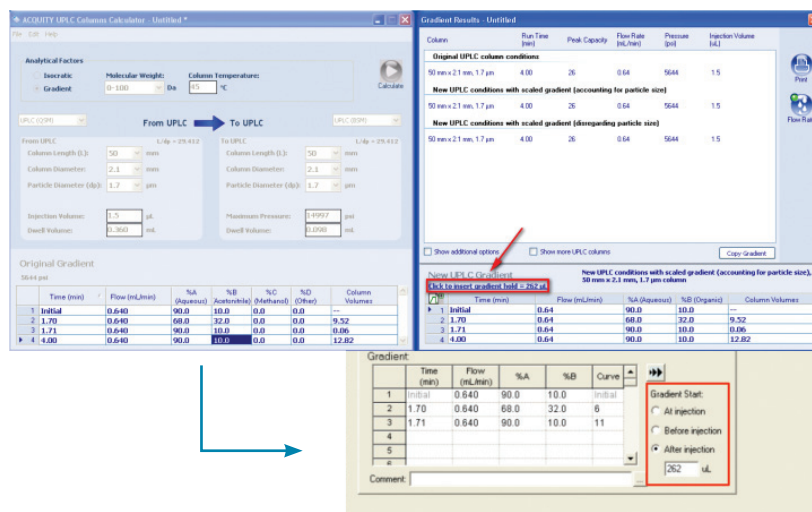


Figure 1. a) Columns Calculator used to calculate method conditions for separation on ACQUITY UPLC I-Class System; b) New Gradient Start feature in ACQUITY UPLC I-Class BSM instrument method editor.

To demonstrate this new tool, the gradient separation of a series of barbiturates was transferred from the ACQUITY UPLC H-Class System to the ACQUITY UPLC I-Class System. Both of these systems use the flow-through needle (FTN) injector design and have active pre-heating. Therefore, it is not necessary to consider injection delivery and heating differences. Inherently, the ACQUITY UPLC H-Class System with its low-pressure quaternary solvent blending has higher system volume compared to the high-pressure, binary solvent blending of the ACQUITY UPLC I-Class System. If the system volume differences between the two systems are not addressed, the separation looks like that shown in Figure 2. Although the peaks are well resolved, the changed elution profile can affect processing of the results and may result in loss of peaks.

To compensate for the gradient delay differences, the system volumes of both systems were measured. These values and the gradient method were input into the ACQUITY UPLC Columns Calculator which suggested a gradient insert hold of 262 μ L. This value was then entered into the Gradient Start table in the control software's instrument method editor, as shown in Figure 1. The separation run using this approach is shown in Figure 3. Resolution values (both the peak widths and the distance between the peak centers) remain conserved when transferring the method between these two systems.

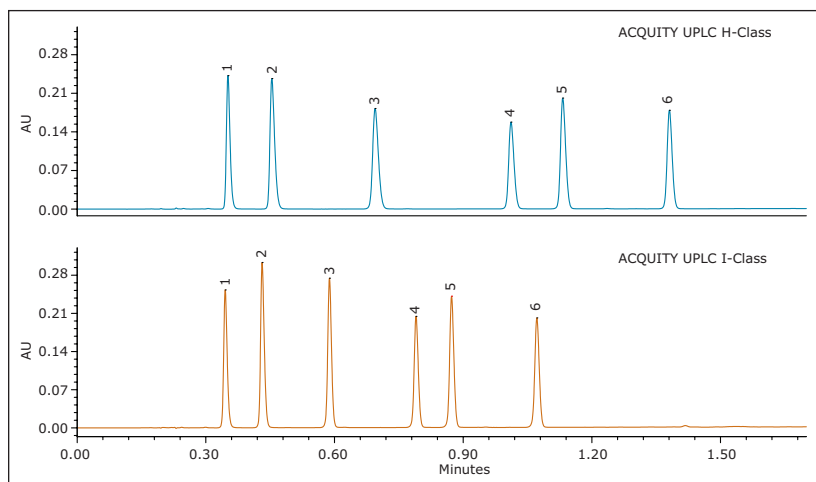


Figure 2. Method transferred from the ACQUITY UPLC H-Class System to the ACQUITY UPLC I-Class System without compensating for differences in system volume.

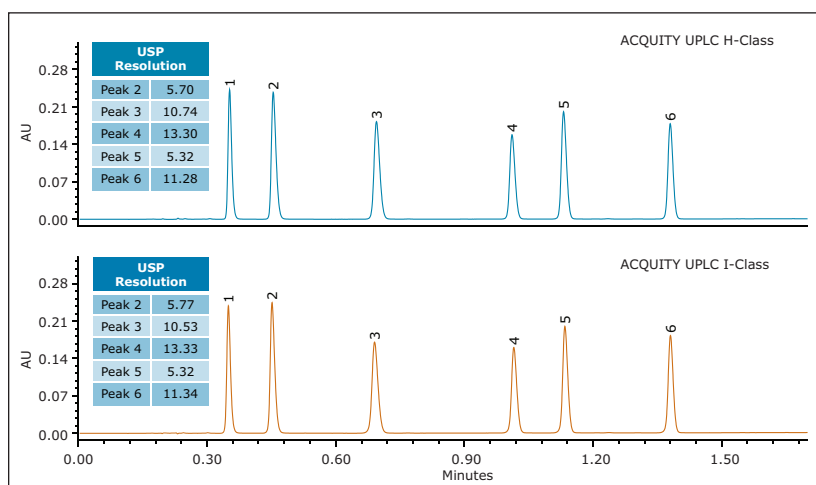


Figure 3. Method transferred from the ACQUITY UPLC H-Class System to the ACQUITY UPLC I-Class System, after accounting for differences in system volume, results in the same elution profile and preserves resolution between peaks.

SUMMARY

Methods developed on the ACQUITY UPLC H-Class System are easily transferred to the ACQUITY UPLC I-Class System with the help of the ACQUITY UPLC Columns Calculator and the new functionality of the Gradient Start feature in the instrument method editor. These enhanced features allow for transfers in both directions, i.e. going from systems with large dwell volumes to small and vice versa. This functionality allows labs with different goals to select the system that best fits their needs without worrying about system differences when transferring methods.

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July 2011 720004029EN LB-PDF

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