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Transferring Compendial HPLC Methods to UPLC Technology for Routine Generic Drug Analysis

Kenneth J. Fountain

Waters Corporation

Abstract

UPLC Technology can provide tremendous benefits to routine testing of generic drug products, including increased sample throughput and decreased solvent consumption (including subsequent disposal).The goal of this document is to educate the reader on how to properly transfer HPLC methods found in the USP-NF (and other Pharmacopeias) to UPLC Technology in order to realize the full benefits of higher throughput, lower costs, and faster time-to-market for routine analysis of generic drugs.

Introduction

Manufacturers of generic drugs must be able to prove that the compounds they produce are comparable to the brand-name drug while maintaining and/or improving the cost effectiveness of the drug's production. While the analytical methodologies used to characterize many drugs can be both difficult and costly to develop, generic drug producers can refer to the United States Pharmacopeia-National Formulary (USP-NF),[1] as well as the British, European, and Japanese Pharmacopeias for information on how to analyze drug substance, drug product, and associated excipients using various analytical techniques such as high performance liquid chromatography (HPLC).

UPLC Technology enables the use of sub-2-µm particle columns with smaller inner diameters (≤ 3.0 mm) and short lengths (50 to 100 mm) on low dispersion, high pressure instrumentation. As a result, lower flow rates and shorter run times are used, which reduces solvent and sample consumption and disposal. The same resolution can be achieved in less time with UPLC, which saves the time and money associated with method development and routine analysis of drug substances and drug products.[2]

The goal of this document is to educate the reader on how to properly transfer HPLC methods found in the USP-NF (and other Pharmacopeias) to UPLC Technology in order to realize the full benefits of higher throughput, lower costs, and faster time-to-market for routine analysis of generic drugs. While each application focuses on a particular drug product (tablets, capsules, ointments, and oral suspensions), the process for transferring HPLC methods found in the USP-NF to UPLC follows a standard procedure, which be reviewed and explained so that the reader can adapt the approach for any USP monograph in existence today. The reader will understand how to select a modern chemistry equivalent based on the "L" designation in the USP-NF, transfer the HPLC separation to UPLC, and understand the practices necessary to ensure

that the transferred method can be used routinely in a production quality control (QC) laboratory. The main goals of this document are to demonstrate that compendial methods can be transferred to UPLC Technology in three easy steps, and supply the reader with the best practices for using UPLC for method development and routine analysis of generic drug formulations.

Results and Discussion

Method Transfer in Three Easy Steps

The following three steps are used to transfer an existing USP method to UPLC Technology (Figure 1):

1. A Waters HPLC column with the appropriate "L" designation is selected and the USP method is run on this column.
2. The HPLC method was transferred to a UPLC column with the same resolving power and stationary-phase chemistry but smaller particle size using the ACQUITY UPLC Columns Calculator.
3. A routine use study is performed using the UPLC column to evaluate the suitability of the method for use in quality control (QC) laboratories.

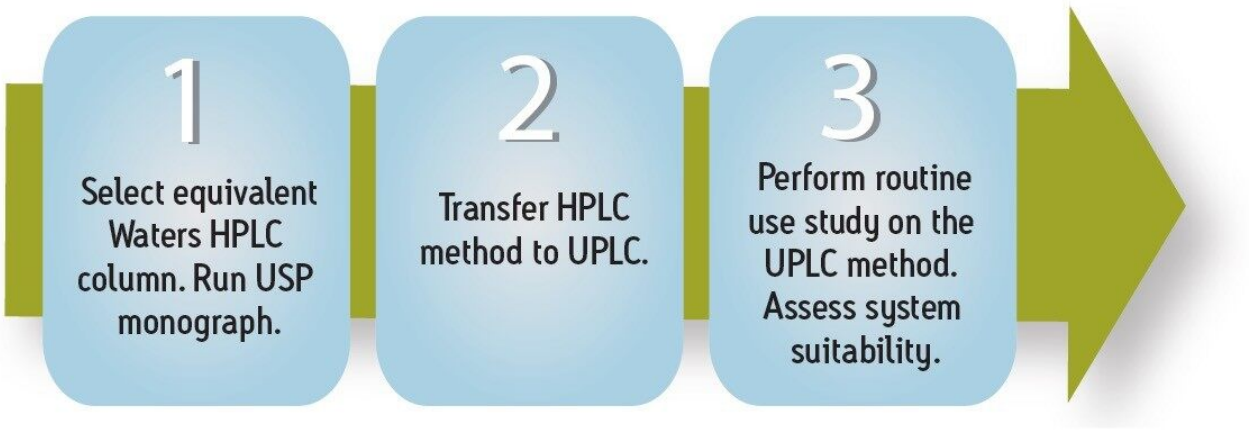

Figure 1. Universal workflow diagram for transferring USP methods from HPLC to UPLC.

The next three sections will briefly describe the details of these steps to ensure successful implementation of

UPLC Technology for generic drug analysis.

HPLC Column Equivalency

Parameters such as base particle chemistry, pore size, and ligand density can make selecting an "equivalent" column difficult for routine analysis. All of the HPLC columns specified for use in the USP monographs can be converted to equivalent Waters columns due to the availability of Waters columns in both HPLC and UPLC particle sizes. This facilitates transfer of HPLC methods to UPLC without a change in selectivity of the separation.

In order to properly choose a Waters HPLC column equivalent to the column type specified in the USP-NF monograph, the Waters Reversed-Phase Column Selectivity Chart can be used at (http://www.waters.com/waters/promotionDetail.htm?id=10048475). This chart plots the hydrophobicity of a column on the x-axis and the relative retention for bases (e.g., selectivity) on the y-axis using a controlled set of conditions. It can be used to compare columns from many different vendors to determine which columns have similar selectivity to each other.

For example, in the USP monograph for ziprasidone HCl,[3] the suggested column designation is "L7" which is a C_8 ligand bonded to porous silica particles. The column used to develop the original USP monograph was an Agilent Zorbax Rx-C8 column. Using the Waters Reversed-Phase Column Selectivity chart, the Waters column with the closest selectivity to the Zorbax column in the L7 category is XBridge C_8 (Figure 2).

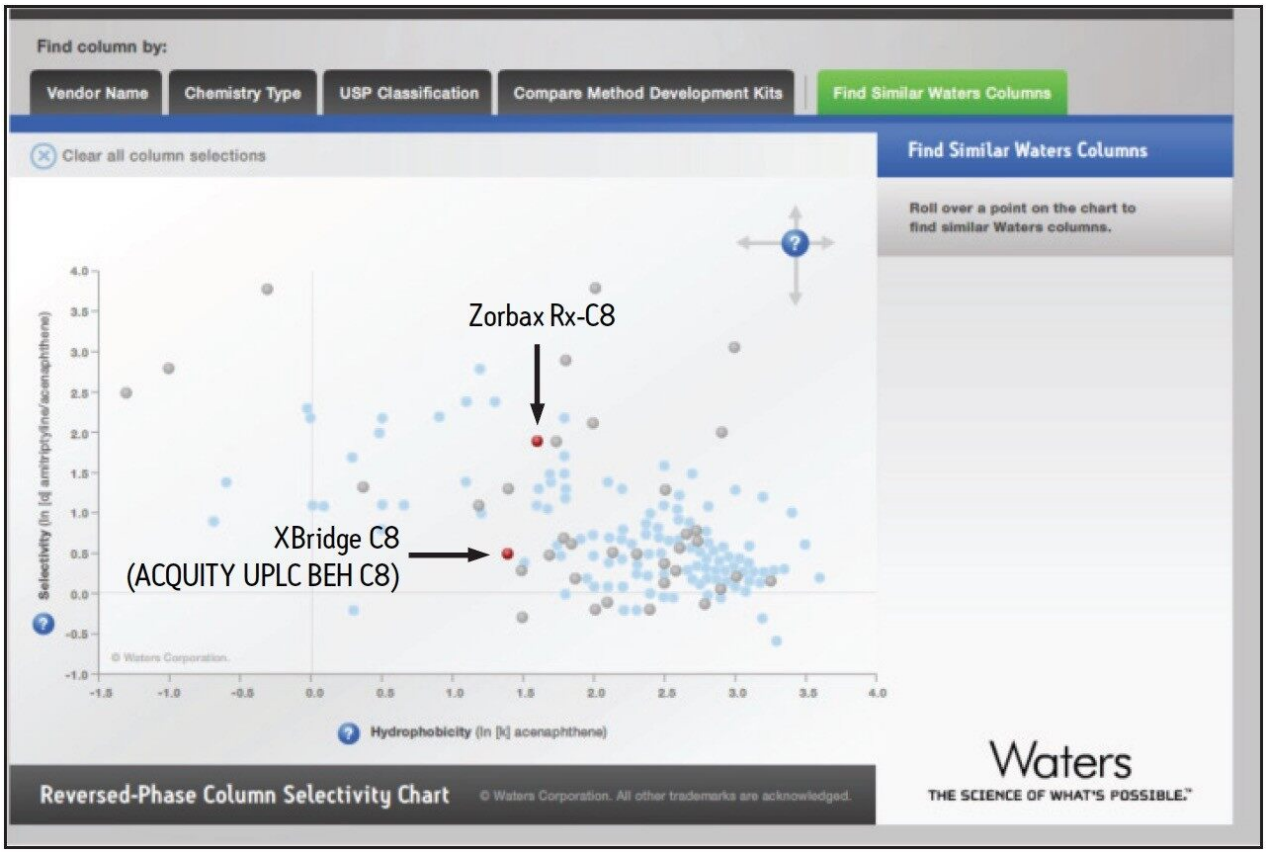

Figure 2. Waters Reversed-Phase Column Selectivity Chart. The XBridge C_8 column is the Watrers equivalent 1.7 column choice to the Zarbox RX-C_8 column specified in the USP monograph for ziprasidone HCL.

Once this is determined, the Waters column is used to run the methodology specified in the USP monograph to ensure that the separation on the Waters column meets the specified system suitability criteria.

HPLC Method Transfer to UPLC

Once the USP monograph for each generic drug is run with a Waters equivalent HPLC column on an HPLC system and the system suitability criteria are met, the separation is then transferred to an equivalent UPLC column on a UPLC system.

In order to properly transfer a method between systems, the following requirements must be met:

1. The selectivity of the original separation must be preserved by using the same column chemistry but in a smaller particle size when transferring from HPLC to UPLC.

2. The resolving power of the original column must be maintained in the new column. This is accomplished by using columns that have the same ratio of column length to particle size ratio (described below).

3. The flow rate, injection volume, and gradient times (if applicable) must be properly scaled from the HPLC system and column to the UPLC system and column in order to preserve the separation selectivity.

To address the first two requirements above, Waters offers Method Transfer Kits that contain an HPLC column and UPLC column that have the same resolving power and chemistry but different particle sizes. The advantage to using smaller particles is that shorter column lengths can be used to achieve the same efficiency (or peak capacity in a gradient).[2] As a result, shorter run times and faster linear velocities can be used (Figure 3) to increase throughput and decrease costs in a routine analysis laboratory. The following column dimensions have the same resolving power, which is calculated from the ratio of the column length (L) to particle size (dp):

HPLC - 150 mm, 5 µm

HPLC - 100 mm, 3.5 µm

HPLC - 75 mm, 2.5 µm

UPLC - 50 mm, 1.7 µm

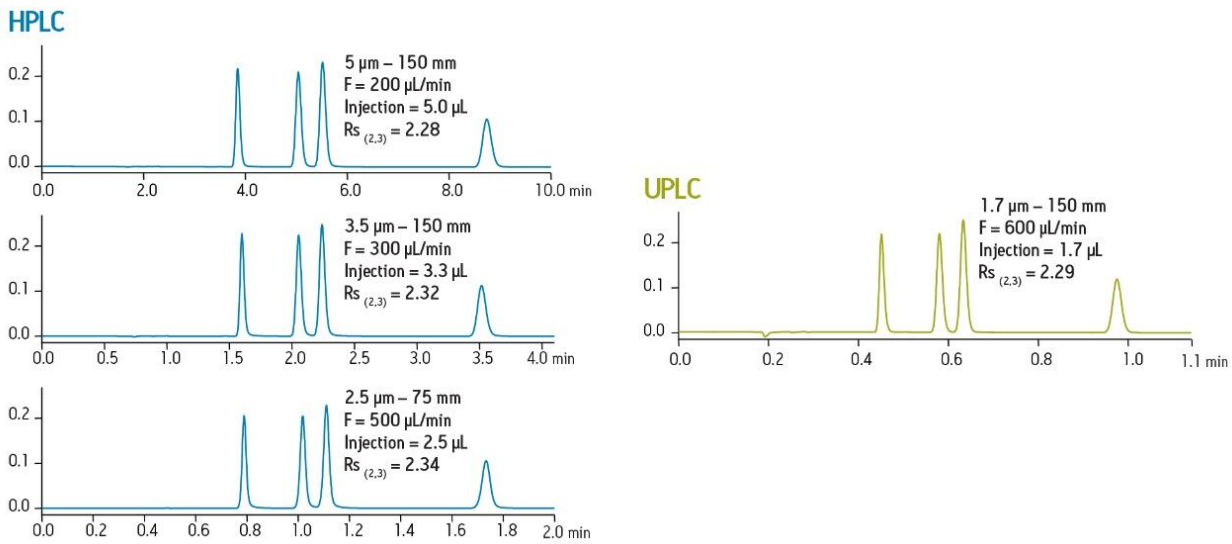

Figure 3. Separation of 1-methylxanthene, 1,3-dimethyluric acid, theobromine, and 1,7-dimethylxanthene (in order of increasing elution) on four different column dimensions with the same resolving power (L/dp ratio).

The third requirement can be achieved using the ACQUITY UPLC Columns Calculator. This calculator allows the transfer of methods from HPLC to UPLC, UPLC to HPLC, and between different HPLC and/or UPLC systems. The original method conditions are entered into the calculator by the user, and the conditions for the new method are automatically displayed into a results window. The resulting method can also be directly

exported to Empower without the need for manual transcription. For gradient separations, the dwell volumes of the two systems should be measured and entered for proper method transfer.

The isocratic method transfer calculations for the USP assay method for levonorgestrel and ethinyl estradiol tablets[4] using the ACQUITY UPLC Columns Calculator are shown in Figure 4. Figure 4A shows the original method conditions on HPLC, and it also indicates the UPLC system to which the method will be transferred. There is a field to select the column dimensions of the UPLC column to ensure that the resolving power (L/dp ratio) is maintained. Figure 4B shows the resulting UPLC method options. One of the options accounts for the change in particle size, which calculates the optimum flow rate based on both the changes in column dimensions and particle size (smaller particles can be operated at faster flow rates without loss in performance). The second option disregards particle size, and only accounts for the column geometry. It is recommended to use the method that accounts for particle size to take full advantage of UPLC for optimal separations.

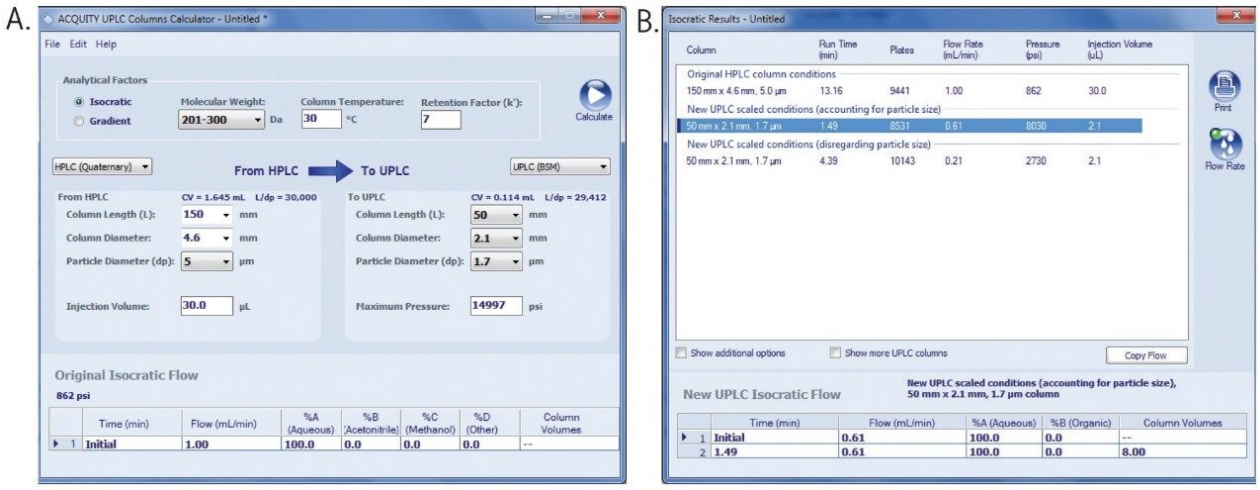

Figure 4. ACQUITY UPLC Columns Calculator for the transfer of the USP assay monograph for levonorgestrel and ethinyl estradiol tablets from HPLC to UPLC. This is an isocratic method.

For gradient separations, the dwell volume of each system should be specified to ensure that the gradient reaches the head of the column at the same time for both the HPLC and UPLC methods, allowing the selectivity of the original separation to be preserved. Figure 5 shows an example of a gradient method transfer from HPLC to UPLC for mometasone furoate ointment.[5] The original HPLC conditions are shown in Figure 5A and the resulting UPLC method is shown in Figure 5B. The resulting UPLC method suggests a pre-injector volume of 62 µL. This number can be entered into the UPLC instrument method in Empower to ensure the sample is injected at the same time the gradient reaches the head of the column, ensuring that

the separation selectivity of the HPLC method does not change. If this field indicates that a hold time is needed, the user has the option to incorporate this into the gradient table for UPLC. Additional information on using the ACQUITY UPLC Columns Calculator can be found in a previously published application note.[6]

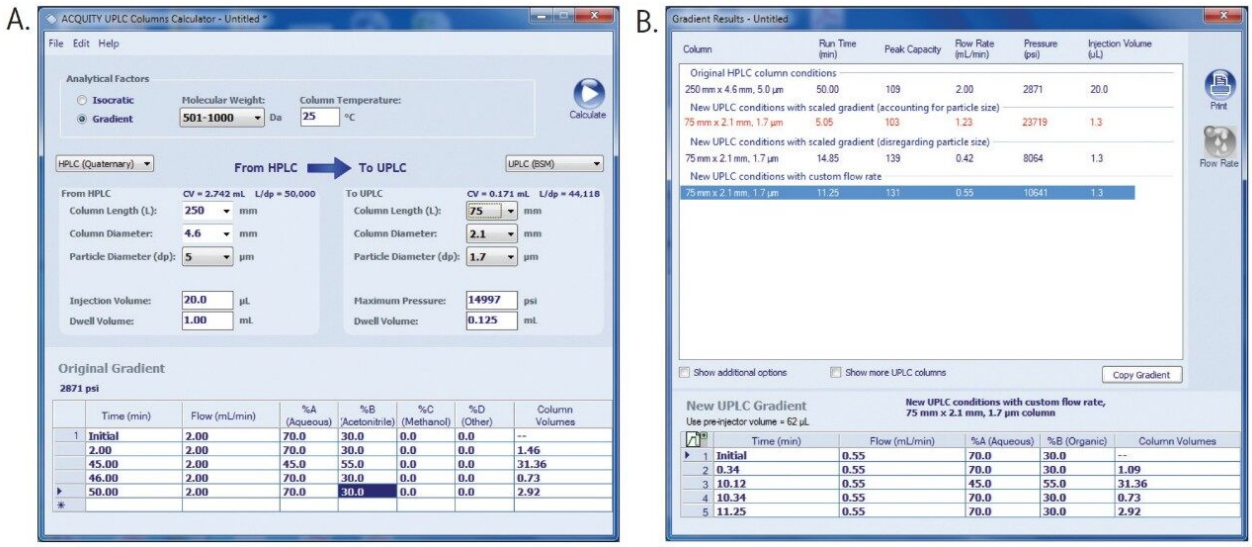

Figure 5. ACQUITY UPLC Columns Calculator for the transfer of the USP assay monograph for levonorgestrel and ethinyl estradiol tablets from HPLC to UPLC. This is an gradient method.

Evaluation of UPLC for Routine Analysis of Generic Drugs

There are some general precautions and best practices that will ensure successful implementation of UPLC for routine analysis of generic drug formulations:

1. Use high quality water. All mobile phases should use in-house purified MilliQ water (18.2 MΩΩ · cm).
2. Filter all buffers to eliminate particulates and other contaminants through a 0.2-µm filter.Mobile phases should be prepared fresh and completely replaced every one or two days to avoid contaminating the UPLC system. In the event that bacterial contamination does occur, a full system cleaning is recommended before performing further work.
3. When appropriate, filter samples through a 0.2-µm syringe filter to remove particulates.
4. Consider centrifuging samples at a higher speed and/or for longer time to more efficiently removeparticulates.
5. Use 0.2-µm pre-column filters (part number 205000343) to protect the UPLC column from particulates.

For methods that have potentially problematic formulation components and cannot be properly filtered prior to analysis, VanGuard Pre-Columns (2.1 x 5 mm UPLC guard columns) are recommended.

6. Perform a water/organic or high organic wash regularly, either as part of the sample set, as a daily protocol, or after analysis of a batch of samples.
7. Columns should be stored in 100% organic, preferably acetonitrile, especially when they will not be used for an extended period of time. It is important to remember that the mobile phases used for generic drug analysis should be properly flushed with miscible water or water/organic mixtures prior to storage in 100% organic solvent.

Conclusion

UPLC Technology can provide tremendous benefits to routine testing of generic drug products, including increased sample throughput and decreased solvent consumption (including subsequent disposal). Analysis times can be reduced by 70 to 90%, and solvent consumption can be decreased by over 90%. This offers significant cost benefits associated with running more samples in less time, and can decrease the overall operating expenses in development and quality control laboratories. Most of all, it helps provide a competitive advantage to generic drug companies who leverage UPLC Technology to bring drugs to market faster and cheaper.

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