應用手冊

Modernization of the Acetaminophen USP Monograph Gradient HPLC Method for Impurities using USP <621> Guidelines and MaxPeak™ Premier HPS Technology

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Abstract

USP monographs articulate the quality expectations of a medicine including for its identity, strength, and purity. They also describe the tests to validate that a medicine or its ingredients meet these criteria. The test methods found within the monograph are validated and can be used for the analysis of various pharmaceutical products ranging from ophthalmic solutions to oral dosage formulations. These methods, while suitable, often use older HPLC columns that employ large particles (***e.g.*** 5 µm) packed into long columns, resulting in long analysis times and high mobile phase usage. Using modern columns packed with smaller particles can significantly reduce the analysis time and the volume of mobile phase consumed, leading to savings in both time and money.

A USP monograph method utilizing a gradient separation was modernized following the USP <621> guidelines. The original liquid chromatography method, which is designed for the analysis of organic impurities in acetaminophen, utilizes a 4.6 x 250 mm, 5 µm column, and a 73 min gradient. After modernization to a MaxPeak Premier High Performance Surfaces (HPS) 4.6 x 150 mm, 2.5 µm Column, the method only requires a 36 min gradient. All system suitability requirements were met while achieving significant reductions in both analysis time and solvent consumption.

Benefits

- Comparable chromatographic results with modernized method conditions
- 51% reduction in analysis time using modernized conditions
- 40% reduction in solvent usage

Introduction

United States Pharmacopeia (USP) monographs contain validated test methods designed for specific formulated products which are available to consumers either over the counter or prescribed as part of a medical treatment. Because these methods have already been validated, they are more readily adopted by testing laboratories than are methods developed from scratch. This is especially important for generic pharmaceutical manufacturers, where releasing product quickly to market is paramount. While these methods may be easily adopted, they often employ older column technology that includes wide bore columns packed with 5 µm particles. Due to the low efficiency per unit length of these types of columns, analysis times are often long. However, with advancements in column technology, specifically the use of smaller particles packed in shorter columns, these long analysis times can be reduced.

The greatest benefits of modernization can be seen when moving to sub-2 µm particle size columns. However, these columns require LC systems which can operate at high pressures and have low dispersion. Modernizing older HPLC methods to 2.5 µm columns still provides significant benefits but does not require the highest performing LC systems. Modernizing USP monograph methods, if not done properly, can require costly re-validation. There are processes and guidelines which provide insights into modernization as outlined in general chapter <621> of the USP. These guidelines cover the allowable changes to parameters like flow rate, column dimensions and particle size. In December 2022, the USP <621> guidelines were revised to allow for the modernization of gradient methods and include the necessary calculations to ensure the gradient profile of the new testing conditions matches the original conditions. The calculations outlined in <621> are complex, which can lead to mistakes. Tools like the Waters™ Column Calculator provide the new conditions without the potential for miscalculations.

This application note demonstrates the use of the Column Calculator to modernize the organic impurities analysis of acetaminophen from the original monograph method to a method employing a UHPLC column packed with 2.5 µm particles. The system suitability criteria for both the original method, as well as the modernized version were

tracked to ensure both adhered to the assay requirements. The original method requires a 4.6 x 250 mm 5 µm L7 column and a 73 min run time per sample. The modernized method uses a 4.6 x 150 mm 2.5 µm column and a 36 min run time. Calculations of the solvent and time savings were performed after the analyses to highlight the benefits of modernizing this assay.

Experimental

Sample Preparation

Three separate solutions were created as outlined in the USP monograph method. The system suitability standard contained 20 µg/mL acetaminophen, and 80 µg/mL each of both Related Compound B and C in methanol. The standard solution contained 1.25 µg/mL of Related Compound D and 0.25 µg/mL Related Compound J in methanol. The sample solution contained 25 mg/mL of acetaminophen in methanol.

LC Conditions

LC systems:	1260 Infinity LC with UV Detector (monograph method) ACQUITY Arc™ with 2998 PDA (modernized method)
Detection:	UV @ 254 nm
Columns:	Zorbax Eclipse Plus C_8, 5 µm, 4.6 x 250 mm (monograph method) XBridge™ Premier BEH C_8, 2.5 µm, 4.6 x 150 mm (modernized method)
Column temp.:	40 °C

Sample temp.:	10 °C
Injection volume:	5.0 µL (monograph method) 3.0 µL (modernized method)
Flow rate:	0.9 mL/min (monograph method) 1.1 mL/min (modernized method)
Mobile phase A:	Methanol:Water:Glacial Acetic Acid (50:950:1)
Mobile phase B:	Methnaol:Water:Glacial Acetic Acid (500:500:1)
Gradient conditions:	Table 1

Time (min) – original method	Time (min) – modernized method	%A	%B
0.00	0.00	82	18
8.00	3.93	82	18
53.00	26.02	0	100
58.00	28.47	0	100
59.00	28.96	82	18
73.00	35.94	82	18

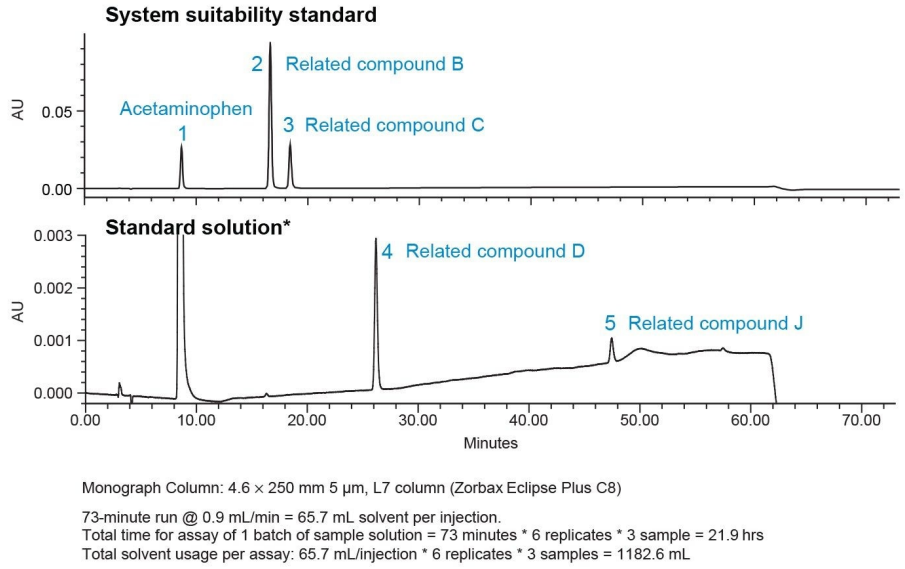
Table 1. Gradient profile for both original monograph and modernized methods.

Data Management

Chromatography software:	Empower™ 3 Feature Release 4

Results and Discussion

The original method for organic impurities of acetaminophen is outlined in the USP monograph.[1] System suitability criteria for the method include USP tailing, resolution, and peak area relative standard deviation measurements. The USP tailing factor for related compound D cannot be more than 2.0. The USP resolution must be not less than 2.0 between acetaminophen and related compound B, and not less than 1.5 between related compound B and related compound C. The peak area relative standard deviation for related compound D must be less than 5.0%.[1] These conditions were first tested using an Agilent 1260 Infinity LC system and a 4.6 x 250 mm, 5 µm Zorbax Eclipse C_8 (L7 designation) column. Representative chromatograms for each standard injected are shown in Figure 1. Not shown is the sample solution, which was injected but has no system suitability criteria associated with it.



USP tailing factor R.C.-D	**1.16**
USP resolution R.C.-B	**21.20**
USP resolution R.C.-C	**4.31**
%RSD retention time R.C.-D	**0.40%**
%RSD peak area R.C.-D	**0.82%**

All criteria pass

Monograph Column: 4.6 × 250 mm 5 µm, L7 column (Zorbax Eclipse Plus C8)

73-minute run @ 0.9 mL/min = 65.7 mL solvent per injection.
Total time for assay of 1 batch of sample solution = 73 minutes * 6 replicates * 3 sample = 21.9 hrs
Total solvent usage per assay: 65.7 mL/injection * 6 replicates * 3 samples = 1182.6 mL

Figure 1. Chromatograms showing separations of organic impurities of acetaminophen using the USP monograph conditions with a 4.6 x 250 mm, 5 µm Zorbax Eclipse C_8 column and an Agilent 1260 Infinity LC system.

A good separation was achieved for all components of the system suitability and standard solutions. It should be noted that acetaminophen was added to the standard solution to ensure accurate gradient performance between the standard and system suitability solution. Typically, acetaminophen would not be present in the standard

solution. The USP tailing factor for related compound D was 1.16, less than the system suitability limit. The USP resolution for acetaminophen and related compound B was 21.2, well above the required 2.0 minimum. The USP resolution for related compounds B and C was 4.31, again well above the requirement of no less than 1.5. Lastly the relative standard deviations for related compound D were 0.40% for retention time and 0.82% for peak area, both less than 5.0% which is the requirement for this test. With all system suitability criteria met, a batch of material could be tested to determine the concentrations of organic impurities.

In order to test a batch of material using these conditions a total of 18 injections are needed. Six replicates of each standard and the sample solution are required as the relative standard deviations required in this monograph are greater than 2.0%.[2] Given the run time of 73 min per injection and 18 injections this means that using the USP monograph method as written, a single batch of material would require 21.9 hour of run time on the instrument. At a flow rate of 0.9 mL/min the total testing would require 1183 mL of mobile phase. These are not insignificant numbers as a full calendar day of testing is required to release a batch. Not only that, but if something happens to the system or column in hour 21 of testing, then the entire day of testing is wasted, as is the mobile phase consumed during that time.

Using the Waters Column Calculator and USP <621> guidelines this monograph can be modernized to a suitable UHPLC column to reduce both the analysis time and the solvent consumption. Prior to any actual testing however, an appropriate UHPLC column must be selected. The USP monograph specifies that an L7 column must be used. USP <621> allows changes to column configuration so long as the L/dp (length to particle size ratio) stays within -25% to +50% of the original monograph column. A 250 mm column packed with 5 µm particles has an L/dp ratio of 50,000. When modernizing to a 2.5 µm particle column, in order to maintain that ratio a 150 mm column was used. A similarly packed 100 mm column would also be acceptable with an L/dp of 40,000. The modernized column has an L/dp ratio of 60,000 which is well within the guidelines set by <621>. An XBridge Premier BEH C_8 4.6 x 150 mm 2.5 µm Column was selected for modernization as it employs a robust particle paired with MaxPeak Premier High Performance Surface (HPS) Ttechnology. The XBridge BEH C_8 stationary phase is based on fully porous hybrid-silica particles with a trifunctionally bonded C_8 ligand, placing it into the L7 designation. The XBridge BEH C_8 stationary phase is the most modern L7 fully porous column and is scalable from 5 µm down to sub-2 µm particles.

Now that an appropriate column configuration haCos been selected, the monograph conditions must be modernized considering the new column internal diameter, length, and particle size. These include method flow rate, injection volume, gradient profile, and run time. General Chapter <621> outlines the calculations required for this modernization, however for this work the Waters Column Calculator was used. The Column Calculator uses

the same formulas to calculate new method conditions as outlined in <621> but does so with only minimal input from the user. Figure 2 shows the column calculator, filled in with the original method conditions, as well as the new modernized column information. Additionally, system dwell volume measurements are included.

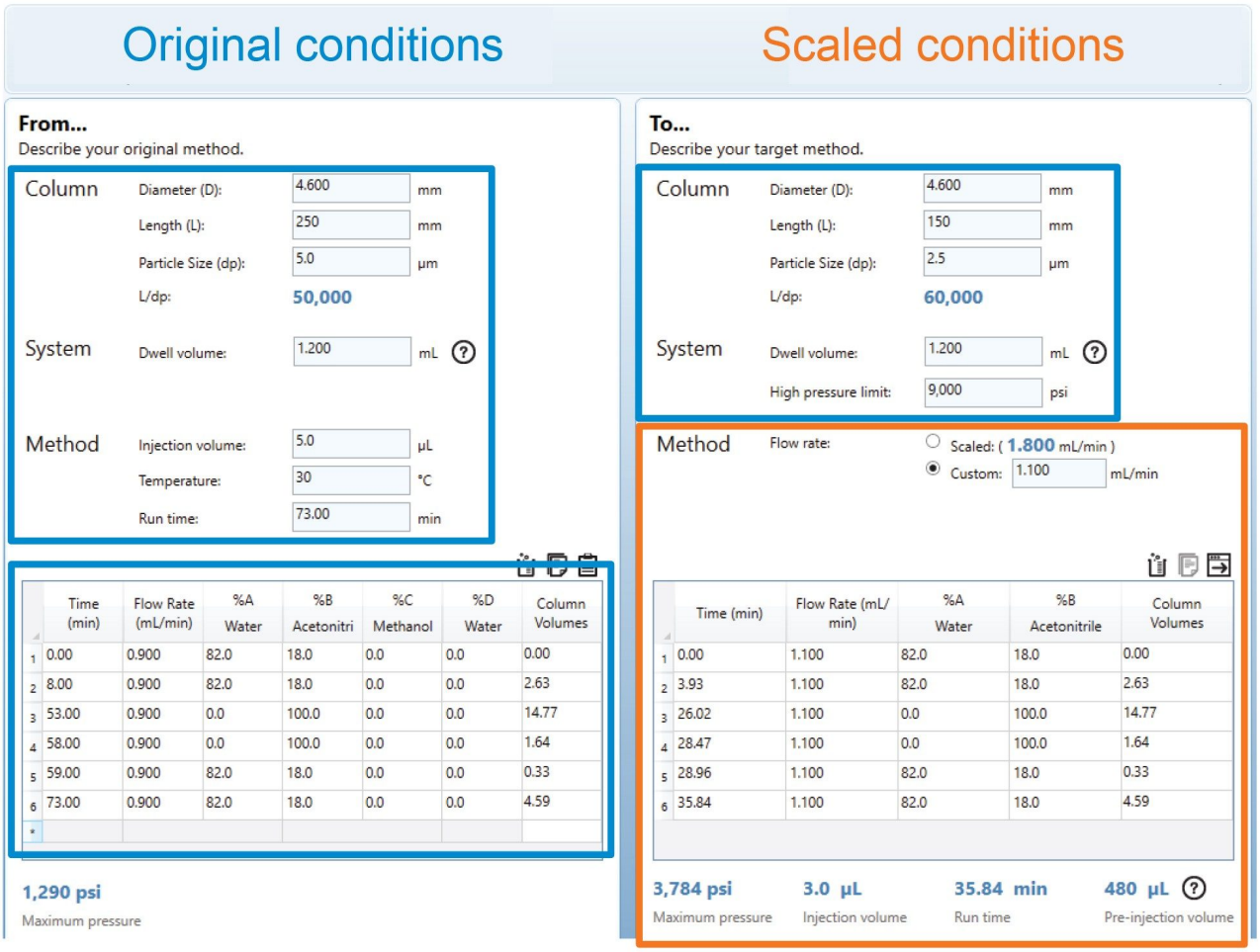
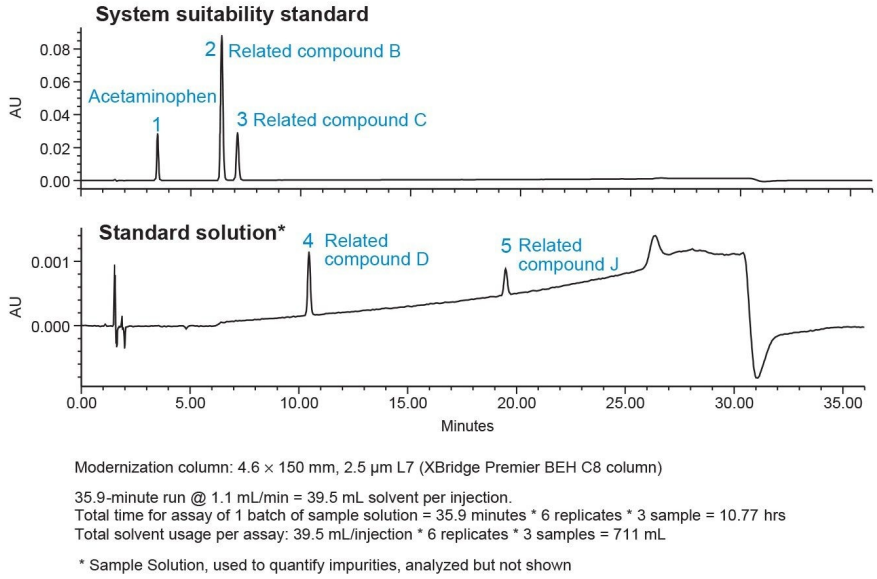


Figure 2. Screenshot from the Column Calculator used to modernize the USP monograph method for organic impurities of acetaminophen.

The areas highlighted in blue require user input, including the original column dimensions, particle size, flow rate, system dwell volume, injection volume, temperature, and run time. Additionally for gradient assays, the gradient profile must be input on the bottom left of the calculator so that the new gradient can be calculated. Lastly, on the top right section, the modernized column information must be input, along with the new system dwell time

and high-pressure limit. The area highlighted in orange is calculated based on the user inputs. The scaled flow rate, in this case 1.8 mL/min is based on the original flow rate, and column dimensions. A custom flow rate can also be set, if the scaled flow rate creates too much pressure for the system being used. In this case a custom flow rate of 1.1 mL/min was selected as the scaled flow rate caused the system to over pressurize. Based on the custom flow rate selected, and the column dimensions of both the original and modernized conditions, the new gradient profile can be calculated. At the bottom right-hand side, we can see the new gradient profile, along with important information such as injection volume, and total run time. These conditions were used to analyze the same samples as before, but on an ACQUITY Arc System with the column previously discussed. The ACQUITY Arc System is well designed for 2.X µm particles as it has lower system dispersion compared to HPLC systems. The representative chromatograms in Figure 3 show separations of both the system suitability and standard solutions using these new conditions.



USP tailing factor R.C.-D	**1.08**
USP resolution R.C.-B	**17.04**
USP resolution R.C.-C	**3.57**
%RSD retention time R.C. -D	**0.03%**
%RSD peak area R.C.-D	**0.50%**

All criteria pass

Modernization column: 4.6 × 150 mm, 2.5 µm L7 (XBridge Premier BEH C8 column)

35.9-minute run @ 1.1 mL/min = 39.5 mL solvent per injection.
Total time for assay of 1 batch of sample solution = 35.9 minutes * 6 replicates * 3 sample = 10.77 hrs
Total solvent usage per assay: 39.5 mL/injection * 6 replicates * 3 samples = 711 mL

* Sample Solution, used to quantify impurities, analyzed but not shown

Figure 3. Chromatograms showing separations of organic impurities of acetaminophen, using the modernized conditions with a 4.6 x 150 mm, 2.5 µm XBridge Premier BEH C_8 Column and an ACQUITY Arc System.

From first glance, the chromatography produced using the modernized method conditions is comparable to the original conditions. Looking more closely at the system suitability results confirms that the modernized conditions are appropriate for this assay. The USP tailing factor was 1.08 for related compound D. The USP resolutions were 17.0 and 3.5 for acetaminophen/related compound B and related compound B/C respectively.

The relative standard deviations for related compound D were 0.03% and 0.50% for retention time and peak area, respectively. With these results, all system suitability criteria were met. Additionally, we have reduced the overall run time from 73 to 36 min per injection. With the modernized conditions, a full test would require just under 11 hours of run time, compared to 22 hours with the original testing conditions. Mobile phase usage is also reduced, with the modernized conditions only requiring ~700 mL compared to the original which needed ~1180 mL. A detailed comparison of the time and mobile phase savings is shown in Table 2.

Conditions	Column dimension	Flow rate (mL/min)	Cycle time/ injection (min)	mL Mobile phase/ injection	mL Solvent per batch	Total analysis time per batch (min/hr)	%Reduction in solvent usage (mL)	%Reduction in analysis time per batch (hr)
Original USP	4.6 × 250 mm 5 µm	0.9	73.0	65.7	1182.6	1314.0/21.9	–	–
Modernized	4.6 × 150 mm 2.5 µm	1.1	35.9	39.5	711.0	646.2/10.8	40%	51%

Table 2. Comparison of time and mobile phase usage between the original and modernized methods.

The modernization of this one assay can save a significant amount of analysis time and solvent. Combining modernization efforts across multiple assays in a laboratory would further increase the savings. By modernizing to UHPLC columns instead of UPLC, sub-2 µm particle columns, the method can be run on conventional HPLC systems which are not rated for the extreme pressures generated by sub-2 µm particle columns. XBridge Premier columns are a good platform for future-proofing analytical methods, as the family includes a wide range of particles sizes, suitable for HPLC, UHPLC, or UPLC analyses. The scalability of the particles allows for easier modernization of older USP monograph methods. By using XBridge Premier columns and the Columns Calculator, modernization of USP monograph methods can be done smoothly with minimal rework.

Conclusion

USP methods are widely used for testing various pharmaceutical products. Some of these methods, especially those for products which have been on the market for a long time, use older column technology. Newer technology, including smaller particle size columns can provide similar results while reducing analysis times and mobile phase usage. LC methods found within USP monographs can be modernized to newer technology by

following the guidelines in USP general chapter <621> which is facilitated by using tools like the Columns Calculator to accurately determine the new method conditions.

Here we demonstrated the modernization of a gradient HPLC method, for the organic impurities assay of acetaminophen. The monograph method uses a 4.6 x 250 mm, 5 µm column, requiring almost an entire day of run time and over a liter of mobile phase to test a single batch. Modernization to a 4.6 x 150 mm, 2.5 µm XBridge Premier BEH C_8 Column was performed, reducing the run time by 51% and mobile phase usage by ~40%. Method modernization may seem like a daunting task, but with the proper tools the process can be performed seamlessly, yielding significant reductions in operating costs.

References

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