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TECHNICAL REPORT

TITLE: MODERNIZING THE USP ORGANIC IMPURITIES METHOD FOR ACETAMINOPHEN BY IMPLEMENTING HALO® COLUMN TECHNOLOGY

MARKET SEGMENT: PHARMACEUTICAL

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ABSTRACT

With the updated Chapter <621> guidance from the United Stated Pharmacopeia (USP), changes are now allowed to be made for HPLC gradient methods. Many monographs could benefit from modernization since they specify large particle sizes such as 5 µm, 4.6 mm ID, long length columns that consume both large quantities of solvent and time. By switching to Fused-Core® HALO® columns, these methods can be shortened, which will reduce mobile phase consumption all without the need for revalidation. Taking the monograph method for the organic impurities in acetaminophen and translating it to a HALO® C8 column reduces the solvent consumption by 45% as well as the time by up to 70%.

INTRODUCTION

Acetaminophen, or paracetamol as it is known outside of the United States, is one of the most used pharmaceuticals in the world [1]. Since it is available over-the-counter (OTC), it is easy to access and is prescribed for every age group from children to adults. The market value for acetaminophen at the end of 2022 was US$ 9.8 billion with the predicted market value to be US$ 15.2 billion by the year 2033 [2]. Additionally, acetaminophen is used in combination with other OTC medicines for the treatment of cold and flu symptoms. With so many people using acetaminophen, the analysis of the levels of impurities present in each batch is a crucial step in its production.

The USP monograph for the organic impurities in acetaminophen, which was official as of January 1, 2023, specifies a 73-minute gradient method using a fully porous particle (FPP) 5 µm, 4.6 x 250 mm C8 column. Since there are three samples that must be run 6 times each, this equates to 18 injections that are required for a batch to be tested. The total time for all of these experiments to be run is 21.9 hours and the total mobile phase required is 1183 mL. Since USP has updated its guidance in Chapter <621>, changes to the particle type, size, and column dimension are allowed without the need for revalidation. The same L classification column must be maintained so in this case a L7 or C8 column must be selected. The process for changing methods consists of calculating -25% to +50% of the length to particle size ratio (L/dp) of the monograph column, adjusting the flow rate

KEY WORDS:

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based on the particle type and size, and adjusting the gradient program and injection volume. The L/dp ratio for the monograph column used for the organic impurities in acetaminophen is 50,000, which means that the range for L/dp can be 37,500 to 75,000. A HALO 90 Å C8, 2.7 µm, 4.6 x 150 mm column has a L/dp of 55,556 which meets the L/dp criteria. The calculations for changing flow rate, gradient time program, and injection volume may be done either by hand using the equations in Chapter <621> or by using an online method translation calculator.

EXPERIMENTAL:

The examples in this report will demonstrate time and solvent savings for the organic impurities in acetaminophen using modernized methods based on the USP monograph for acetaminophen and the updated Chapter <621> guidance from USP. All solvents used were HPLC grade. Methanol and glacial acetic acid were obtained from MilliporeSigma (St. Louis, MO). Acetaminophen standards were obtained from MilliporeSigma (St. Louis, MO).

Column: HALO 90 Å C8, 2.7 µm, 4.6 x 150 mm
Part Number: 92814-708
Mobile Phase A: 50:1:950 Methanol, glacial acetic acid, water
Mobile Phase B: 500:1:500 Methanol, glacial acetic acid, water
Flow Rate: 1.67 mL/min (maximize time savings) or
1.3 mL/min (reduced pressure)
Pressure: 262 bar (1.67 mL/min)
231 bar (1.3 mL/min)
Temperature: 40 °C
Detection: UV 254 nm, PDA
Injection Volume: 2.8 µL
Sample Solvent: Methanol
Data Rate: 100 Hz
Response Time: 0.025 sec.
Flow Cell: 1 µL
Instrument: Shimadzu Nexera X2

Gradients:

Flow Rate: 1.67 mL/min		Flow Rate: 1.3 mL/min	
Time:	%B	Time:	%B
0.00	18	0.00	18
2.38	18	3.05	18
15.74	100	20.18	100
17.22	100	22.08	100
17.52	18	22.47	18
21.68	18	27.80	18

RESULTS:

Since not all laboratories have access to UHPLC systems, the organic impurities in acetaminophen method was translated using two different flow rates: 1.3 mL/min for reduced pressure and 1.67 mL/min to maximize speed. One advantage of superficially porous particles compared to fully porous is the ability to increase the flow rate and maintain column efficiencies due to the core/resistance to mass transfer. Using these two flow rates, the back pressure was maintained below 400 bar. With sustainability in mind, the original monograph method was not run since it would consume nearly 22 hours of instrument time and ~1200 mL of mobile phase. Table 1 shows the solvent and time reductions using the modernized methods. Using 1.3 mL/min, the solvent is reduced by 45% and the time is reduced by 62%. When 1.67 mL/min is used, the solvent is also reduced by 45%, but the time is reduced by 70% compared to the original monograph method.

Column	FPP C8, 5 µm, 4.6 x 250 mm	HALO®C8, 2.7 µm, 4.6 x 150 mm	HALO® C8, 2.7 µm, 4.6 x 150 mm
Time (min)	73	*27.8*	*21.68*
Flow Rate (mL/min)	0.9	*1.3*	*1.667*
Total Volume/ Batch (mL)	1183	*651*	*651*
Total Time/ Batch (hours)	21.9	*8.3*	*6.5*
% Reduction in Solvent (mL)	NA	*45*	*45*
% Reduction in Time (hours)	NA	*62*	*70*

Table 1. Solvent and time savings for the modernized methods for USP organic impurities in acetaminophen.

The monograph method specifies suitability requirements for the system suitability solution as well as the standard solution. Figure 1 shows the results for the system suitability solution run at both flow rates.

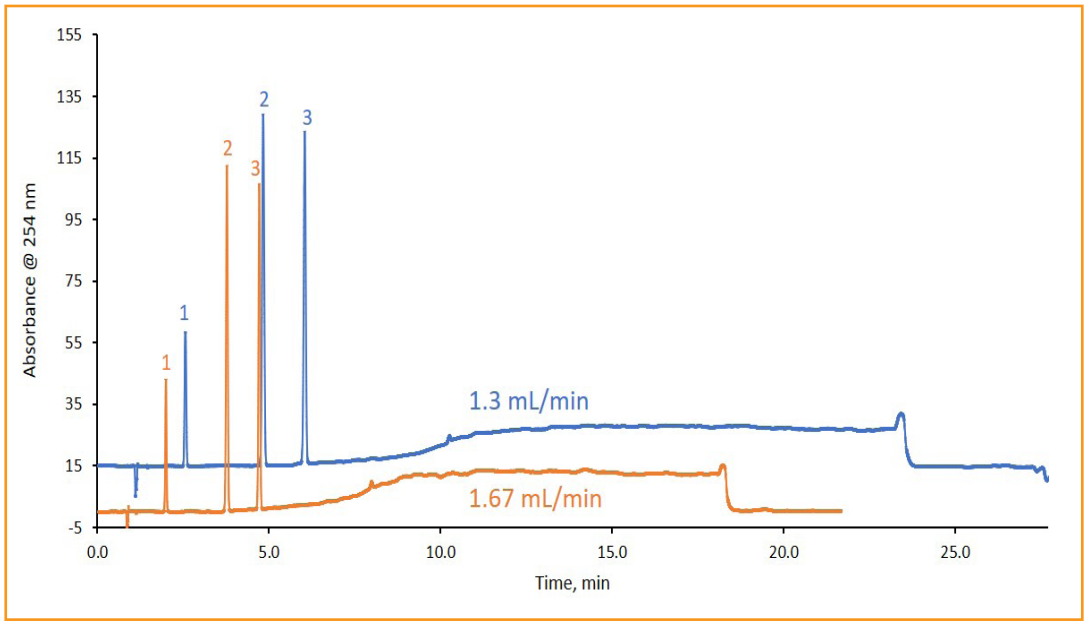

Figure 1. Modernized method for USP organic impurities in acetaminophen showing the system suitability solution run at 1.3 mL/min and 1.67 mL/min on a HALO® C8, 2.7 µm 4.6 x 150 mm column. Peak identities in order are acetaminophen (1), acetaminophen related compound B (2), and acetaminophen related compound C (3).

The suitability requirements for resolution are easily met using both methods. See Table 2 for the results.

	1.67 mL/min method	1.3 mL/min method
NLT 2.0 Resolution between acetaminophen and acetaminophen related compound B	22	22
NLT 1.5 Resolution between acetaminophen related compound B and acetaminophen related compound C	11	11

Table 2. Resolution values for the modernized method for system suitability for the USP organic impurities in acetaminophen.

The standard solution results are shown in Figure 2, which include acetaminophen related compound D and acetaminophen related compound J. The concentrations were increased to 100 µg/mL for acetaminophen related compound D and 20 µg/mL for acetaminophen related compound J for demonstration purposes.

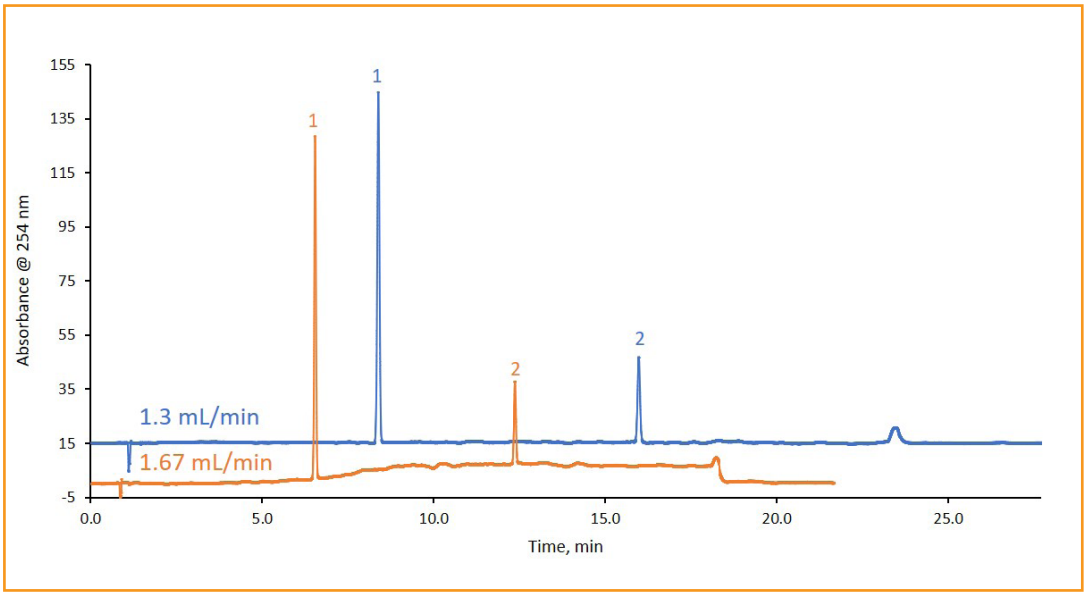

Figure 2. Modernized method for USP organic impurities in acetaminophen showing the standard solution run at 1.3 mL/min and 1.67 mL/min on a HALO® C8, 2.7 µm 4.6 x 150 mm column. Peak identities in order are acetaminophen related compound D (1) and acetaminophen related compound J (2).

The suitability requirements are met using both methods. See Table 3 for the results. The peak shapes are symmetrical and the % RSD values for both retention time and peak area are well within the specified criteria of the method.

	1.67 mL/min method	1.3 mL/min method
NMT 2.0 Tailing factor for acetaminophen related compound D	1.02	1.02
NMT 5% RSD for acetaminophen related compound D (retention time)	0.01%	0.01%
NMT 5% RSD for acetaminophen related compound D (area)	0.31%	0.48%

Table 3. Tailing factor and % RSD values for the modernized method for the standard solution for the USP organic impurities in acetaminophen.

CONCLUSION:

By modernizing the USP method for organic impurities in acetaminophen, both the time and solvent needed to complete batch testing for impurities is significantly reduced. The method was moved to a smaller particle size, shorter length HALO® C8 column according to the criteria and steps specified in Chapter <621> of the USP. The ability to modernize USP methods aids laboratories who are focusing on becoming more sustainable and environmentally friendly with the added bonus of cost savings since the time and solvent have been reduced compared to the original monograph method conditions.

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