Application Note

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Modernizing the USP Acetaminophen and Caffeine Tablets HPLC Method Following the Revised USP <621> Guidelines

Realizing the benefits of smaller particle size columns without revalidation

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Abstract

The original United States Pharmacopeia (USP) Acetaminophen and Caffeine Tablets HPLC assay was modernized to take advantage of smaller particle columns, including totally porous particle (TPP) and superficially porous particle (SPP) columns, following the newly revised USP <621> guidelines. The updated methods replaced conventional 5 µm TTP columns with 3.5 µm Agilent ZORBAX Eclipse Plus C18 columns and 4 µm Agilent InfinityLab Poroshell 120 EC-C18 columns without revalidation. All system suitability requirements were met and significant reductions in both analysis time and solvent consumption were achieved.

Introduction

Because most USP monographs use HPLC methods for quality control, they are routinely used by pharmaceutical manufacturers. These methods mostly employ old column technology such as conventional 5 or 10 µm TPP columns. Due to their low efficiency, longer columns are often required, leading to long analysis times and high solvent consumption. Therefore, there are needs to modernize the existing methods to take advantage of new column technologies, including smaller and superficially porous particle technologies. Also, analysts need to modernize their existing USP methods without making significant changes that would require revalidation. The new version of USP <621> guidelines that became effective in December 2022 allows laboratories to transfer their isocratic and gradient methods from conventional TPP columns to both TPP columns and SPP columns.[1]

In this application note, per the current USP <621> guidelines, a USP acetaminophen and caffeine tablets isocratic method[2], which used 4.6 × 100 mm, 5 µm columns, was adjusted to allow the use of smaller particle size 3.5 µm ZORBAX Eclipse Plus C18 columns and 4 µm InfinityLab Poroshell 120 EC-C18 columns.

Experimental

Instruments and materials

An Agilent 1260 Infinity II LC system with 0.12 mm tubing throughout was used to evaluate the columns. The instrument configuration is listed in Table 1.

Table 1. Instrument configuration.

Agilent 1260 Infinity II LC system	
Agilent 1260 Infinity II Binary Pump (G7112B)	4-pos/10-port valve, 600 bar (p/n 5067-4287)
Agilent 1260 Infinity II Multisampler (G7167A)	Vial, screw top, amber with write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716) Cap, screw, blue, PTFE/red silicone septa, 100/pk (p/n 5182-0717)
Agilent Infinity II Multicolumn Thermostat (MCT)	Standard flow heater (G7116-60015) Heater and column: InfinityLab Quick Connect assembly, 0.12 × 105 mm (p/n 5067-5957)
Agilent 1260 Diode Array Detector (DAD) WR (G7115A)	Standard flow cell 10 mm, 13 µL (p/n G1315-60022) Long-life deuterium lamp (p/n 2140-0820)
Agilent OpenLab CDS, Version 2.8	

All reagents and solvents were HPLC grade. Methanol, glacial acetic acid, benzoic acid, acetaminophen, and caffeine were purchased from Anpel Laboratory Technologies (Shanghai, China). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK).

Sample preparation

The internal standard solution and standard stock solution were prepared as described in the USP method.[2] The standard solution used for the system suitability analyses contained 0.1 mg/mL acetaminophen, 0.1 mg/mL caffeine and 0.36 mg/mL of benzoic acid in a solution of methanol and glacial acetic acid (95:5).

LC conditions

The LC conditions used for the original and updated methods are provided in Table 2.

Table 2. LC conditions.

	Original USP Method	Adjusted Method
Column	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902)	– Agilent ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm (p/n 959933-902) – Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm (p/n 695970-902)
Mobile Phase	Methanol, glacial acetic acid, and water (28:3:69)	
Flow Rate	2 mL/min	The adjusted volumes are shown in Tables 3 and 4
Temperature	45 °C	
Injection Volume	10 µL (2 µL was used for original method)	The adjusted volumes are shown in Tables 3 and 4
Detection	DAD signal 275 nm, ref off 40 Hz	DAD signal 254 nm, ref off 80 Hz

Results and discussion

The original method used an isocratic HPLC separation, and per the revised USP <621> guidelines, there are two options to modernize it. The first is to adjust the method to use smaller particle TPP columns following the steps described in Case Study 1 of the Agilent white paper "Understanding the Latest Revisions to USP <621>."[3] When updating the method, different length and particle size columns can be used if the ratio of column length to particle diameter (L/dp) remains constant or in the range between −25 to +50% of the prescribed ratio.

In this application note, the author chose the ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm column for the original USP method. The modernized method uses a smaller particle 3.5 µm TPP column with the same chemistry as the ZORBAX Eclipse Plus C18, 4.6 × 75 mm column, which has a similar L/dp ratio of 21,000. Another option is to use the InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm SPP column. The 4 µm InfinityLab Poroshell 120 particles provide double the efficiency of 5 µm totally porous particles. Thus, an InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm column delivers efficiency similar to the ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm column.

The flow rate was adjusted because smaller-particle columns require higher linear velocities to obtain the same performance. The particle size was changed, and flow rate was adjusted for both the change in column diameter and particle size using Equation 1.

Equation 1.

$$F_2 = F_1 \times [(dp_1 \times dc_2^2) / (dp_2 \times dc_1^2)]$$

- F_1= flow rate specified in the USP monograph (mL/min)
- F_2= adjusted flow rate (mL/min)
- dc_1= internal diameter (id) of the column specified in the USP monograph (mm)
- dc_2= id of the column used (mm)
- dp_1= particle size specified in the USP monograph (µm)
- dp_2= particle size of the column used (µm)

The adjusted flow rate, in this case 2.9 mL/min for the ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm column and 2.5 mL/min for the InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm column, was determined based on Equation 1. After adjusting for the change in column dimensions, an additional flow rate change of ±50% is permitted. Similar to the author's previous application note[4], a smaller particle size (sub-2 µm) is not recommended for the method because the high flow rate after adjustment for column dimension can cause column or instrument over pressurization.

The injection volume was adjusted based on Equation 2.

Equation 2.

$$V_2 = V_1 \times [(L_2 \times dc_2^2) / (L1 \times dc_1^2)]$$

- V_1= injection volume specified in the USP monograph (µL)
- V_2= adjusted injection volume (µL)
- L_1= column length specified in the USP monograph (cm)
- L_2= new column length (cm)
- dc_1= column id specified in the USP monograph (mm)
- dc_2= new column id (mm)

The original USP monograph method uses a 10 µL injection, which results in a distorted peak shape due to solvent effects. Therefore, a reduced injection volume of 2 µL was used for the original method. The injection volumes used on the modern columns were determined using Equation 2. The column selection criteria and the HPLC parameters are shown in Tables 3 and 4.

Five replicates of standard solution were analyzed on each column using the parameters in Tables 3 and 4. System suitability requirements evaluated for the method included USP tailing factor, resolution, and peak area relative standard deviation (RSD). The USP tailing factor must not exceed 1.2 for each analyte peak. The USP resolution must not be less than 1.4 between any of the analyte and internal standard peaks. The peak area RSD for each analyte must be less than 2.0%. All these system suitability criteria were met for both the TPP and SPP modernized columns (Table 5.) The chromatograms obtained using the ZORBAX Eclipse Plus C18 and InfinityLab Poroshell 120 columns are shown in Figure 1.

Table 3. Method adjustments for TPP columns.

Column Dimension	L/dp Ratio	Allowable L/dp Range (–25 to +50%)	Flow Rate (mL/min)	Injection Volume (µL)	Column Temperature (°C)	Detector
Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm	20,000	22,500 to 45,000	2	2	45	UV 275 nm, 40 Hz
Agilent ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm	21,429	Meets	2.9	1.5	45	UV 275 nm, 80 Hz

Table 4. Method adjustments for SPP columns.

Column Dimension	Plate Number (N)			Allowable L/dp Range (–25 to +50%)			Flow Rate (mL/min)	Injection Volume (µL)	Column Temperature (°C)	Detector
	N1	N2	N3	N1	N2	N3				
Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm	4,565	4,988	6,866	3,424 to 6,848	3,741 to 7,482	5,150 to 10,299	2	2	45	UV 275 nm, 40 Hz
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm	4,004	4,559	6,013	Meets	Meets	Meets	2.5	1	45	UV 275 nm, 80 Hz

Table 5. System suitability test results.

System Suitability Requirements	Resolution (R) (≥ 1.4 between any of the analyte and internal standard peaks)		Tailing Factor (≤ 1.2 for each analyte peak)			%RSD of Peak Area (n = 5) (≤ 2.0%)		
	R Between Peak 1 and 3	R Between Peak 2 and 3	Peak 1	Peak 2	Peak 3	Peak 1	Peak 2	Peak 3
Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm	25.1	19.7	1.09	1.06	1.04	0.77	0.84	0.89
Agilent ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm	27.4	21.5	1.12	1.08	1.06	0.98	0.18	0.12
Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm	20.7	16.8	1.16	1.07	0.97	0.47	0.37	0.52

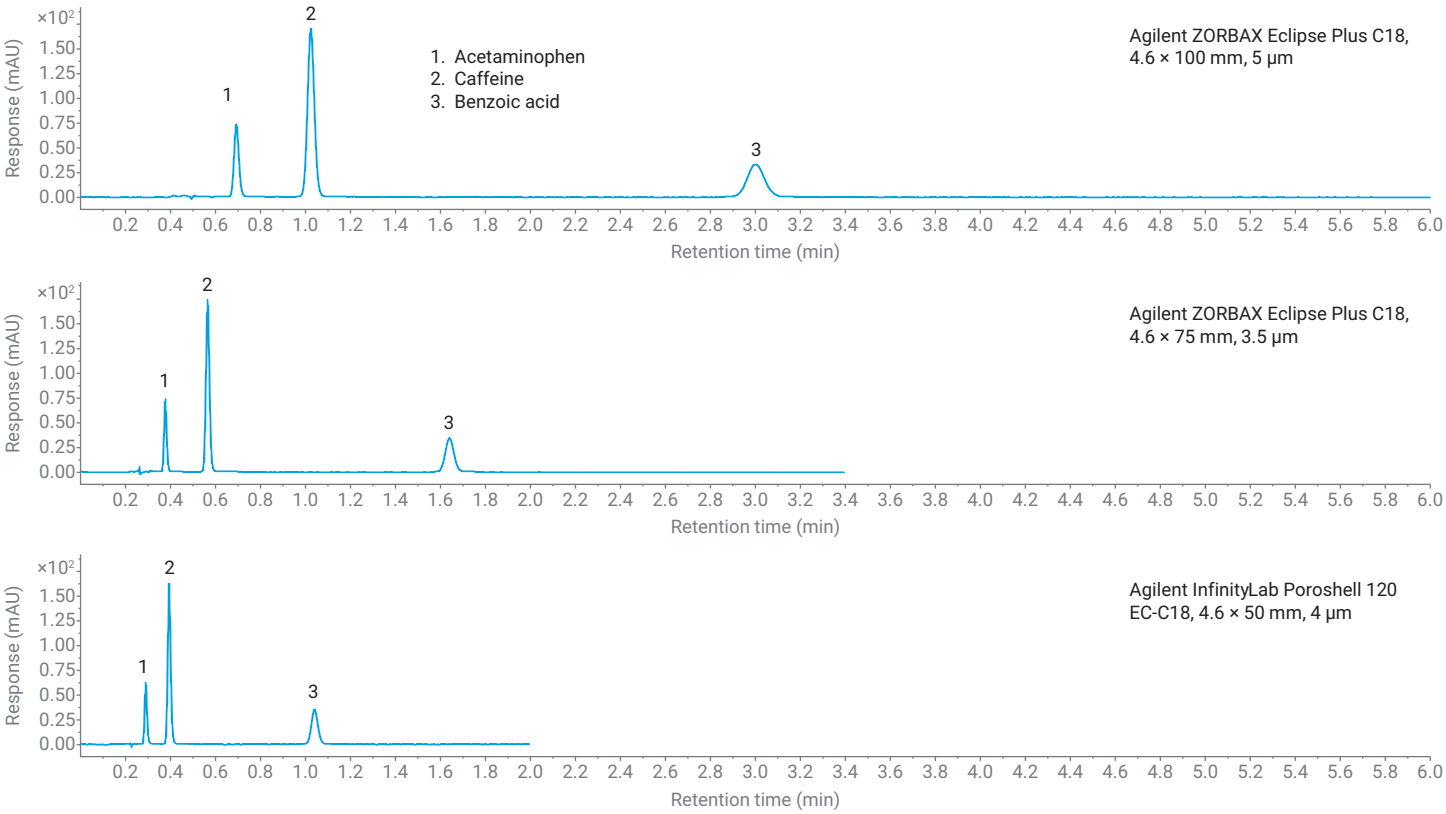

Figure 1. Chromatograms obtained from analysis of the USP acetaminophen and caffeine tablets system suitability solution for the different length and particle size columns evaluated.

Compared to the original methods, overall method run time and solvent consumption were reduced when using the smaller particle columns evaluated, as shown in Table 6. In this study, a 4.6 id column was used for modernized methods that require high flow rates. Narrower id columns, such as 3.0 mm id columns, are highly recommended to further reduce solvent consumption.

Assay modernization can save substantial analysis time and solvent. Both the ZORBAX Eclipse Plus C18 and InfinityLab Poroshell 120 EC-C18 columns are good platforms for method transfer, as these families of columns include a wide range of particle sizes and column dimensions suitable for HPLC and UHPLC analyses. The scalability of particle sizes allows for modernization of older USP monograph methods quickly, easily, and with minimal rework.

Table 6. Comparison of the analysis time and mobile phase consumption for the original and modernized methods.

Method	Column Dimension	Flow Rate (mL/min)	Analysis Time/ Injection (min)	Mobile Phase Consumption/ Injection (mL)	Solvent Saved (%)	Analysis Time Saved (%)
Original Method	Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm	2.0	4	8	–	–
Modernized Methods	Agilent ZORBAX Eclipse Plus C18, 4.6 × 75 mm, 3.5 µm	2.9	2	5.8	27.5	50
	Agilent InfinityLab Poroshell 120 EC-C18, 4.6 × 50 mm, 4 µm	2.5	1.2	3	62.5	70

Conclusion

USP monograph methods that use older column technology can be modernized to newer column technology by following the guidelines provided in USP general chapter <621>. Use of newer column technology, including smaller particle size and SSP columns, can provide similar results while reducing analysis times and mobile phase consumption.

This application note demonstrated the adaptation of an isocratic HPLC method for the USP Acetaminophen and Caffeine Tablets assay from older to more modern column technology. Specifically, a method that used a conventional 4.6 × 100 mm, 5 µm column was modernized to take advantage of Agilent ZORBAX Eclipse Plus C18 and Agilent InfinityLab Poroshell 120 columns of various particle sizes and dimensions, without the need for revalidation. The revised methods met system suitability requirements and provided reductions in both analysis time and solvent consumption.

References

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