Method Development for the Analysis of USP Acetaminophen and Caffeine Tablets

Agilent ZORBAX Eclipse Plus and Poroshell 120 columns meet USP requirements

Application Note

Small Molecule Pharmaceuticals

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Abstract

A method for acetaminophen and caffeine tablets was run on totally porous Agilent ZORBAX Eclipse Plus 5 µm and 3.5 µm columns columns and a superficially porous Agilent Poroshell 120 4 µm column, according to the United States Pharmacopeia (USP) assay for this tablet. The method was modified within the guidelines of USP Chapter 621. All the system requirements were met by the columns.

Introduction

USP recommends traditional 4 µm or 5 µm totally porous columns for HPLC analysis. More recently, superficially porous particles have become popular as HPLC packing materials. Due to a shorter mass transfer distance and narrower particle size distribution, superficially porous particles have higher efficiency than totally porous particles. Methods developed on 5 µm columns can be moved to Agilent Poroshell 120 4 µm columns quickly and easily, with minimum method modification.

This application note describes a method developed for the USP assay of acetaminophen and caffeine tablets [1] with traditional Agilent ZORBAX Eclipse Plus C18 3.5 µm and 5 µm columns, and an Agilent Poroshell 120 EC-C18 4 µm column. The analyses were compared with regard to the chromatographic system requirements of USP.

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Materials and Methods

All reagents and solvents were HPLC or analytical grade. The standards were purchased from USP. Glacial acetic acid and methanol were purchased from J&K Scientific Ltd, Beijing. The standard and assay solutions were prepared according to the USP monograph of acetaminophen and caffeine tablets [1]. The HPLC analysis was performed with an Agilent 1200 Series Rapid Resolution LC (RRLC) system including an:

- Agilent G1312B Binary Pump SL
- Agilent G1376C Automatic Liquid Sampler SL (ALS)
- Agilent G1316B Thermostatted Column Compartment SL (TCC)
- Agilent G1316C Diode Array Detector SL (DAD)

Conditions

Columns:	Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm (p/n 695970-902) Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm (p/n 959996-902) Agilent ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 3.5 µm (p/n 959961-902)
Mobile phase:	Water:methanol:glacial acetic acid (69:28:3)
Temperature:	45 °C
Flow rate:	2.0 mL/min
Injection volume:	2 µL
Detection:	UV, 275 nm

Results and Discussion

The chromatographic system for USP acetaminophen and caffeine tablets requires, "The liquid chromatograph is equipped with a 275 nm detector and a 4.6 mm × 10 cm column that contains 5 µm packing L1" [1]. In this work, we used traditional ZORBAX Eclipse Plus C18 3.5 µm and 5 µm columns and a Poroshell 120 EC-C18 4 µm column, which were all within the USP guidelines.

Figure 1 shows the system suitability analysis for USP acetaminophen and caffeine tablets on three different columns. The top chromatogram shows that the analysis performed as specified by USP with a ZORBAX Eclipse Plus C18, 4.6 × 100 mm, 5 µm column with L1 packing. The mid and bottom chromatograms were run with smaller particle columns, which were still within USP requirements. The 4 µm superficially porous particle column allows quick mass transfer, which leads to weak retention but with a higher efficiency and a larger resolution than a 5 µm totally porous particle column. Compared to a 3.5 µm totally porous particle column, the 4 µm column provided almost the same efficiency, but much more symmetrical peak shapes.

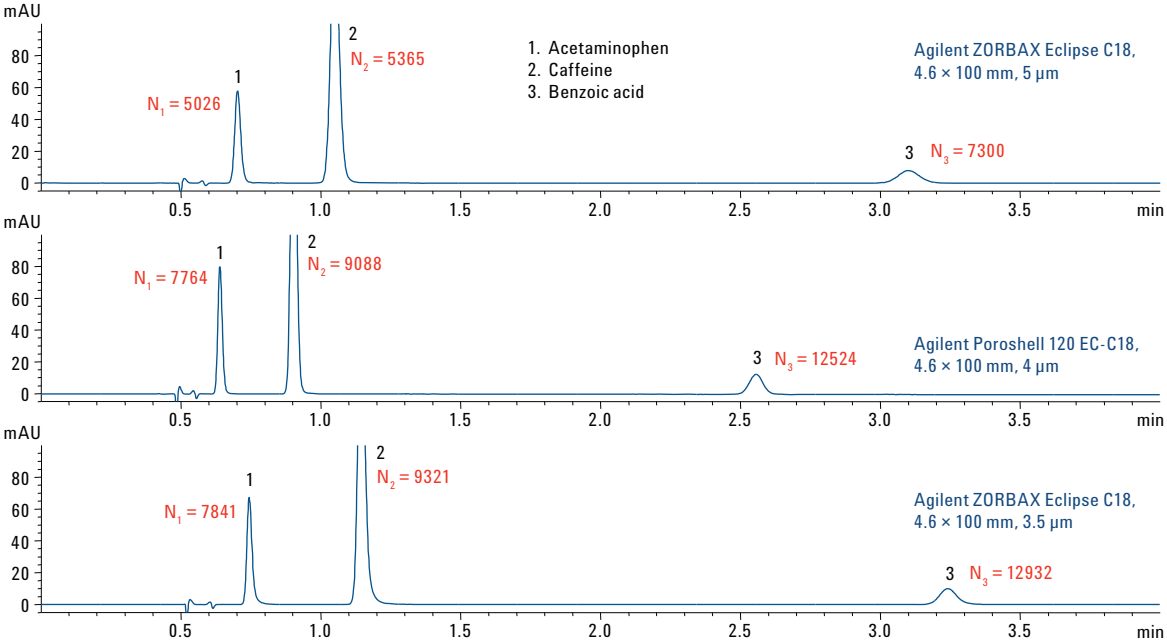

Figure 1. System suitability for USP Monograph: Acetaminophen and Caffeine Tablets assay using Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 columns.

The USP chromatographic system requirements were all measured according to the USP monograph for acetaminophen and caffeine tablets using all columns. Table 1 lists the USP system requirements and measured values on the columns. The methods on the columns met all the USP chromatographic system requirements.

Figure 2 shows an analysis of acetaminophen and caffeine tablets on a Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm column, with benzoic acid as internal standard. The amount of acetaminophen and caffeine could be calculated according to the ratios of the peak responses of the corresponding analyte and internal standard peaks obtained from the sample and the standards, respectively.

Table 1. The USP chromatographic system requirements and measured values for acetaminophen and caffeine tablets.

USP requirements	5 µm column	4 µm column	3.5 µm column
The tailing factor for each analyte peak is not more than 1.2	Tf_1 = 1.13 Tf_2 = 1.09 Tf_3 = 1.06	Tf_1 = 1.12 Tf_2 = 1.08 Tf_3 = 1.00	Tf_1 = 1.19 Tf_2 = 1.18 Tf_3 = 1.11
The resolution, R, between any of the analyte and internal standard peaks is not less than 1.4	$Rs_{1,2}$ = 7.2 $Rs_{2,3}$ = 20.2	$Rs_{1,2}$ = 7.9 $Rs_{2,3}$ = 25.5	$Rs_{1,2}$ = 9.9 $Rs_{2,3}$ = 25.9
The relative standard deviation (RSD) for replicate injections is not more than 2.0%	RSD1 = 0.21% RSD2 = 0.18% RSD3 = 0.14%	RSD1 = 0.16% RSD2 = 0.17% RSD3 = 0.12%	RSD1 = 0.19% RSD2 = 0.16% RSD3 = 0.15%

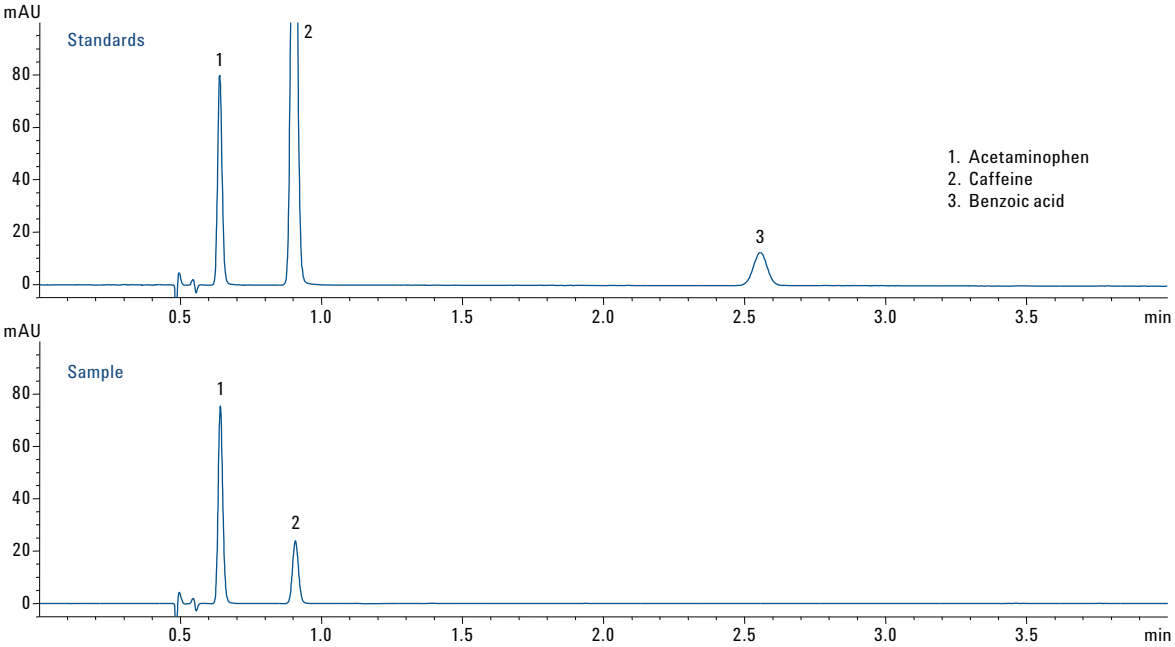

Figure 2. Chromatograms of standards for acetaminophen and caffeine tablets on an Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 4 µm column.

Conclusions

All the methods meet all USP requirements for the chromatographic system. The superficially porous Agilent Poroshell 120, 4 µm column could be successfully substituted for the traditional 5 µm column for the USP assay analysis of acetaminophen and caffeine tablets with high performance and symmetrical peak shapes.

Reference

1. *USP Monograph: Acetaminophen and Caffeine Tablets, USP-35: 2036-2037*. U.S. Pharmacopeial Convention, Rockfield, MD, USA.

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