USP Analysis of Warfarin Sodium Tablets Using Agilent Poroshell 120 EC-CN and SB-C8 Columns

Application Note

Pharmaceuticals

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Abstract

Warfarin, warfarin related compound A, and propylparaben were analyzed according to the United States Pharmacopeia (USP) analysis for warfarin sodium tablets. The chromatographic purity and assay analyses were improved by using superficially porous Agilent Poroshell 120 columns as compared to the USP-suggested 5 µm columns. Each method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 columns. All chromatographic system requirements were met with the improved superficially porous column analyses.

Introduction

There is significant interest in transferring LC methods to superficially porous particles from larger 5 µm totally porous particles. The high efficiency of superficially porous particles is similar to sub-2 µm totally porous particles. This is attributed primarily to a shorter mass transfer distance and a narrower particle size distribution. Furthermore, the larger particle size results in lower backpressure, allowing for these columns to be implemented in methods on virtually any LC system. The benefits of transferring from larger particle columns are very significant time and cost savings, because superficially porous particles are optimally run at faster flow rates and achieve similar resolution with a much shorter column length [1,2].

This application note describes two methods from the USP for the analysis of warfarin sodium tablets that were transferred from suggested 5 µm columns to shorter 2.7 µm superficially porous Agilent Poroshell 120 columns. Each analysis was compared against the USP chromatographic system requirements to ensure column suitability for the analysis. All method modifications are allowable within USP Chapter 621.

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Materials and Methods

The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3]. Analytical method parameters are the same as those specified by the USP.

Warfarin and propylparaben were purchased from Sigma-Aldrich Corp., and warfarin-related Compound A came from the USP. Acetonitrile was purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

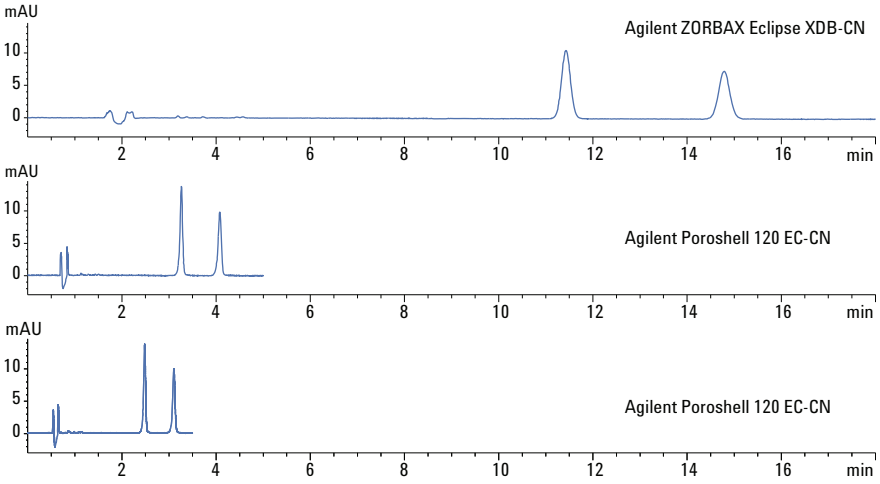
Results and Discussion

USP chromatographic purity analysis of warfarin sodium tablets

Figure 1 shows the USP chromatographic purity analysis for warfarin sodium tablets. The top chromatogram shows the analysis performed as specified by the USP with a 4.6 × 250 mm, 5 µm column with L10 packing, which in this case was a ZORBAX Eclipse XDB-CN column. The two compounds were easily separated in approximately 16 minutes.

Elution order

1. Warfarin (6 µg/mL)
2. Warfarin-related compound A (6 µg/mL)



Conditions

Columns:	Agilent Poroshell 120 EC-CN, 3.0 × 100 mm, 2.7 µm (p/n 695975-305) Agilent ZORBAX Eclipse XDB-CN, 4.6 × 250 mm, 5 µm (p/n 990967-905)
Sample:	Warfarin (6 µg/mL), warfarin related compound A (6 µg/mL)
Eluent:	A = 0.1% CH_3COOH in H_2O B = CH_3OH
Injection volume:	20 µL for 4.6 × 250 mm column, 3.5 µL for 3.0 × 100 mm column
Flow rate:	1.5 mL/min for 4.6 × 250 mm column, 0.64 mL/min or 0.85 mL/min for 3.0 × 100 mm column
Isocratic:	32% B
Temperature:	25 °C
Detector:	260 nm
Instrument:	Agilent 1290 Infinity LC

Figure 1. USP chromatographic purity analysis of warfarin sodium tablets using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns. The bottom chromatogram shows the Agilent Poroshell 120 EC-CN column used at its optimal flow rate.

According to the guidelines in USP Chapter 621, the 4.6 × 250 mm, 5 µm analysis can be transferred to a 3.0 × 100 mm, 2.7 µm Agilent Poroshell 120 EC-CN column, shown in the middle chromatogram of Figure 1. The bottom chromatogram shows the same Poroshell 120 EC-CN column used at its optimal flow rate, which accomplishes the desired separation in 3 minutes.

Table 1 lists the USP chromatographic system requirements and the measured values for each of the three chromatograms found in Figure 1. Not only was the 5 µm column suitable for this analysis, but so was the 2.7 µm Poroshell 120 column. Additionally, the Poroshell 120 column, when used at its optimal flow rate, saved significant time and solvent compared to the original analysis, allowing for increased productivity as well as substantial cost savings.

Table 1. USP chromatographic system requirements and measurements for the chromatographic purity analysis of warfarin sodium tablets (W denotes measured values for warfarin, and A for warfarin-related compound A).

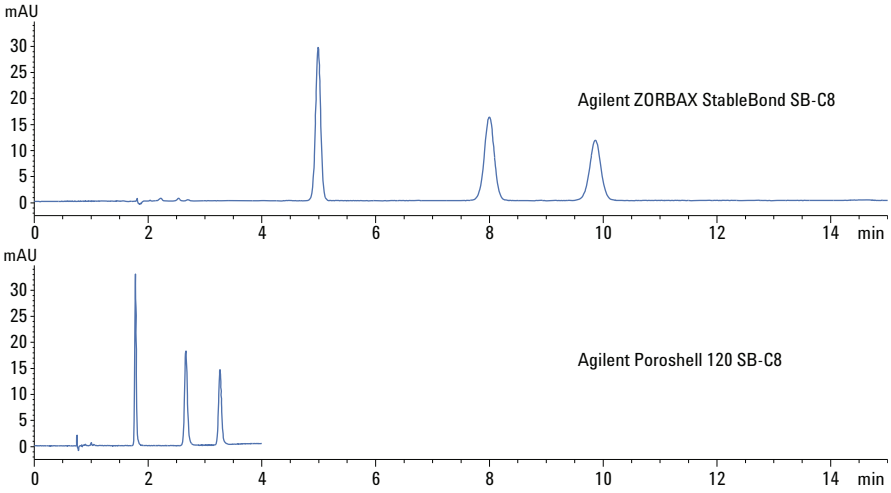
USP chromatographic system requirements	**5 µm (1.5 mL/min)**	**2.7 µm (0.64 mL/min)**	**2.7 µm (0.85 mL/min)**
Resolution between warfarin and warfarin-related compound A is not less than 3	Rs (W,A): 8.3	Rs (W,A): 7.1	Rs (W,A): 7.1
Relative standard deviation for replicate injections is not greater than 5.0%	W: 0.10% A: 0.10%	W: 0.10% A: 0.10%	W: 0.94% A: 0.10%
The relative retention times of warfarin and warfarin-related compound A are 1.0 and about 1.2	W: 1.0 A: 1.3	W: 1.0 A: 1.3	W: 1.0 A: 1.3

USP assay analysis of warfarin sodium tablets

Figure 2 shows the USP assay analysis for warfarin sodium tablets, with propylparaben an internal standard. The top chromatogram is generated using the USP specified 4.6 × 250 mm, 5 µm L7 (ZORBAX StableBond SB-C8) column. The bottom chromatogram is a direct transfer to a 4.6 × 100 mm, 2.7 µm Poroshell 120 SB-C8 column. For this analysis, the Poroshell 120 column could not be used at its optimal flow rate due to pressure limitations. However, regardless of this constraint, the analysis time for these three compounds was still reduced from 10 minutes to less than 3.5 minutes with the Poroshell 120 column.

Elution order

1. Propylparaben (10 µg/mL)
2. Warfarin (5 µg/mL)
3. Warfarin-related compound A (5 µg/mL)



Conditions

Columns:	Agilent Poroshell 120 SB-C8, 4.6 × 100 mm, 2.7µm (p/n 685975-906) Agilent ZORBAX StableBond SB-C8, 4.6 × 250 mm, 5 µm (p/n 880975-906)
Sample:	Warfarin (5 µg/mL), warfarin related compound A (5 µg/mL), propylparaben (10 µg/mL)
Eluent:	A = 0.1% CH_3COOH in H_2O B = CH_3OH
Injection volume:	15 µL for 4.6 × 250 mm column, 5 µL for 4.6 × 100 mm column
Flow rate:	1.4 mL/min
Isocratic:	64% B
Temperature:	30 °C
Detector:	280 nm
Instrument:	Agilent 1290 Infinity LC

Figure 2. USP assay analysis of warfarin sodium tablets using Agilent ZORBAX StableBond SB-C8 and Agilent Poroshell 120 SB-C8 columns.

All chromatograms met the USP chromatographic system requirements, as shown in Table 2. The superficially porous Poroshell 120 column could easily be substituted for the traditional 5 µm column to save costs and improve productivity.

Table 2. USP chromatographic system requirements and measurements for the assay analysis for warfarin sodium tablets (P denotes measured values for propylparaben, W for warfarin, and A for warfarin-related compound A).

USP chromatographic system requirements	5 µm (1.4 mL/min)	2.7 µm (1.4 mL/min)
The relative retention times of propylparaben and warfarin are about 0.75 and 1.0	P: 0.63 W: 1.0	P: 0.67 W: 1.0
Resolution of propylparaben and warfarin is not less than 2.0	Rs (P,W): 12.0	Rs (P,W): 12.5
Relative standard deviation for replicate injections is not greater than 2.0%	P: 0.26% W: 0.26% A: 0.16%	P: 0.10% W: 0.73% A: 0.97%

Conclusions

Superficially porous Agilent Poroshell 120 columns were successfully substituted for traditional 5 µm columns for the USP chromatographic purity and assay analyses for warfarin sodium tablets. Smaller dimension Poroshell 120 columns could be used to improve productivity and to save time and money over larger 5 µm columns, while meeting all USP requirements for the chromatographic system.

References

1. A. Gratzfeld-Hüsgen, E. Naegele. “Maximizing efficiency using Agilent Poroshell 120 columns”. Application Note, Agilent Technologies, Inc. Publication Number 5990-5602EN (2010).

2. V. R. Meyer. *Practical High Performance Liquid Chromatography*, Fourth Ed., p. 34. Wiley (2004).

3. A. Mack. “Optimizing Performance of an Agilent ZORBAX RRHD Eclipse Plus C18 Column by Enhancing an Agilent 1290 Infinity LC System for Ultra-Low Dispersion”. Application Note, Agilent Technologies, Inc. Publication Number 5990-9502EN (2011).

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