USP Analysis of Diphenhydramine and Pseudoephedrine Using an Agilent Poroshell 120 EC-CN Column

Application Note

Pharmaceuticals

Author

Anne Mack

Agilent Technologies, Inc.

Abstract

Diphenhydramine and pseudoephedrine were analyzed according to the United States Pharmacopeia (USP) assay analysis for diphenhydramine and pseudoephedrine capsules. The assay was improved by using a superficially porous Agilent Poroshell 120 column as compared to the USP-suggested 5 µm column. The method was adjusted within the guidelines in USP Chapter 621 to allow for time and solvent savings with the Poroshell 120 column. All chromatographic system requirements were met with the improved superficially porous column.

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 a method from the USP for the analysis of diphenhydramine and pseudoephedrine capsules that was transferred from the suggested 5 µm column to a shorter 2.7 µm superficially porous Poroshell 120 column. The analysis was compared against the USP chromatographic system requirements to ensure column suitability. All method modifications are allowable within USP Chapter 621.

Agilent Technologies

Materials and Methods

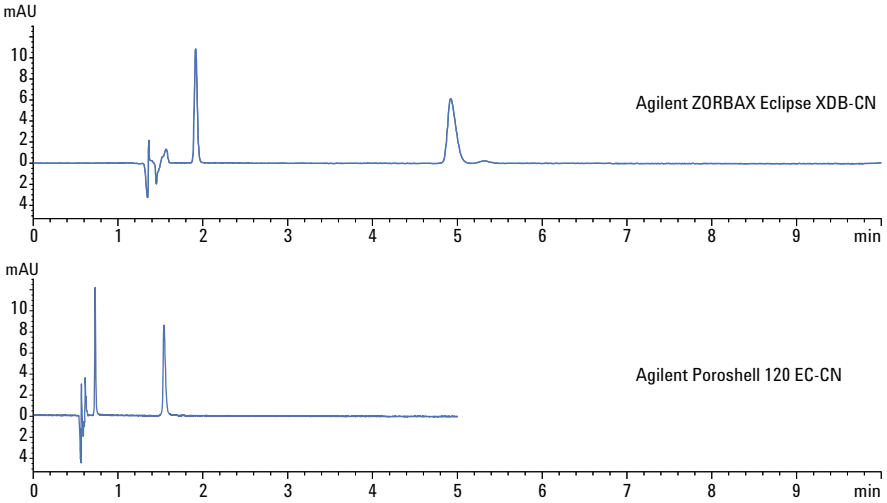
The instrument setup was optimized for lowest possible extra column volume with short 0.075 mm id capillaries found in the Agilent Ultra Low Dispersion Kit (p/n 5067-5189) and with an Agilent LC System Rack (p/n 5001-3726) [3].

Diphenhydramine, pseudoephedrine, 1-heptanesulfonate, glacial acetic acid, and triethylamine were purchased from Sigma-Aldrich Corp. Acetonitrile and methanol were purchased from Honeywell International Inc. Water was 18 Mohm.cm Milli-Q.

Results and Discussion

Figure 1 shows the USP assay analysis for diphenhydramine and pseudoephedrine capsules. 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 about 5.5 minutes.

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 Poroshell 120 EC-CN column, shown in the bottom chromatogram of Figure 1, which accomplished the desired separation in approximately 1.5 minutes.



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:	Diphenhydramine, pseudoephedrine
Eluent:	A = 10 mM $C_7H_{15}NaO_3S$, 13 mM $C_6H_{15}N$, pH 3.3 B = CH_3CN:CH_3OH (26:10)
Injection volume:	20 µL for 4.6 × 250 mm column, 3.5 µL for 3.0 × 100 mm column
Flow rate:	2 mL/min for 4.6 × 250 mm column, 0.85 mL/min for 3.0 × 100 mm column
Isocratic:	36% B
Temperature:	25 °C
Detector:	254 nm
Instrument:	Agilent 1290 Infinity LC

Figure 1. USP assay analysis of diphenhydramine and pseudoephedrine using Agilent ZORBAX Eclipse XDB-CN and Agilent Poroshell 120 EC-CN columns.

Elution order

1. Pseudoephedrine (50 µg/mL)
2. Diphenhydramine (50 µg/mL)

Table 1. USP chromatographic system requirements and measurements for the assay analysis of diphenhydramine and pseudoephedrine capsules (P denotes measured values for pseudoephedrine, and D for diphenhydramine).

USP chromatographic system requirements	5 µm (2 mL/min)	2.7 µm (0.85 mL/min)
Resolution between pseudoephedrine and diphenhydramine peaks is not less than 3.0	Rs (P,D): 24.3	Rs (P,D): 21.2
For each analyte peak the tailing factor is not greater than 2.0	P: 1.2 D: 1.4	P: 1.3 D: 1.5
Relative standard deviation for replicate injections is not greater than 2.0%	P: 0.10% D: 0.82%	P: 0.10% D: 1.9%
The relative retention times are about 1.0 for pseudoephedrine and 3.0 for diphenhydramine	P: 1.0 D: 2.6	P: 1.0 D: 2.1

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

Conclusions

A superficially porous Agilent Poroshell 120 column was successfully substituted for a traditional 5 µm column for the USP assay analysis for diphenhydramine and pseudoephedrine capsules. The smaller dimension Poroshell 120 column could be used to improve productivity and save time and money over the larger 5 µm column, 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).

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
January 7, 2013
5991-1687EN

Agilent Technologies