# Transfer the USP Method for Cephradine from a Traditional 5 µm Column to Poroshell 120

Application Note

Small Molecule Pharmaceuticals and Generics


## Author

Rongjie Fu
Agilent Technologies Co. Ltd
Shanghai, China



## Abstract

A method for cephradine was run on a traditional 5 µm column according to the United States Pharmacopeia (USP) assay for this drug. The method was transferred to a superficially porous Agilent Poroshell 120 EC-C18 Column, which allowed for significant time and solvent savings within the guidelines in USP Chapter 621. The system requirements were all met with the Poroshell 120 EC-C18 Column.


## Introduction

There has been a great deal of interest in transferring LC methods to small particles such as sub-2 µm and 2.7 µm superficially porous particles from 5 µm particles. The 2.7 µm superficially porous particles have high efficiency, similar to that of 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 these columns to be used 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].

This application note describes a method for the USP assay analysis of cephradine [2] using a traditional 5 µm column, which is then transferred to a shorter 2.7 µm superficially porous Poroshell 120 EC-C18 Column. The analyses were compared according to the USP chromatographic system requirements.


Agilent Technologies

## Materials and Methods

All reagents and solvents were HPLC or analytical grade. The standards were purchased from USP. Sodium acetate, acetic acid, and methanol were purchased from J&K Scientific Ltd, Beijing.

The HPLC analysis was performed with an Agilent 1200 Series Rapid Resolution LC (RRLC) including a G1312B Binary Pump SL, G1376C Automatic Liquid Sampler SL (ALS), G1316B Thermostatted Column Compartment SL (TCC), and G1316C Diode Array Detector SL (DAD).

## Results and Discussion

Figure 1 shows the system suitability for USP cephradine analysis. The top chromatogram shows the analysis performed as specified by USP with a ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column with L1 packing. Cephradine and cephalexin were easily separated in 20 minutes, and the resolution of the two compounds was 10.6, which is much greater than the USP requirement of 2.0.

The method was then transferred to a Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column, shown in the bottom chromatogram of Figure 1. The analysis was performed in only 3.5 minutes and the resolution of the two compounds was 9.1, which is still within the USP requirement.

### Conditions

| | |
|---|---|
| Column: | Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)<br>Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902) |
| Mobile phase: | Water:methanol:0.5 M sodium acetate:0.7 N acetic acid (782:200:15:3) |
| Temperature: | 30 °C |
| Flow rate: | 1.0 mL/min |
| Injection volume: | 10 µLfor the 4.6 × 250 mm column,<br>2 µL for the 4.6 × 50 mm column |
| Detection: | UV 254 nm |

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

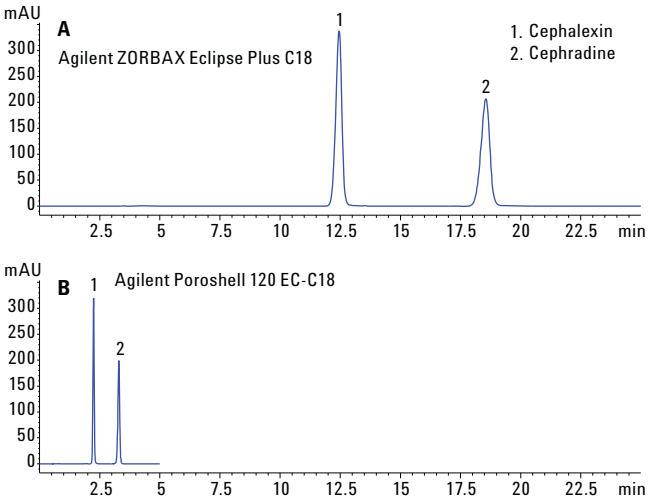


Figure 1. System suitability of USP cephradine analysis using Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 columns.

Table 1. The USP chromatographic system requirements and measured value for cephradine.

| USP requirements | 5 µm column | 2.7 µm column |
|---|---|---|
| Resolution between the cephalexin peak and the cephradine peak is not less than 2.0 | 10.6 | 9.1 |
| The relative retention times are approximately 0.8 for cephalexin and 1.0 for cephradine | Cephalexin: 0.7<br>Cephradine: 1.0 | Cephalexin: 0.7<br>Cephradine: 1.0 |
| The relative standard deviation for replicate injections is not more than 2.0% | 0.12% | 0.14% |

To achieve reliable HPLC results, column-to-column reproducibility is very important. Figure 2 shows chromatograms from different batches of Poroshell 120 columns. The retention time and resolutions of the two compounds demonstrate excellent reproducibility.

## Conclusions

The traditional USP assay for cephradine using a 5 µm column can be successfully transferred to a superficially porous Agilent Poroshell 120 column. The benefits of transferring from larger particle columns include very significant time and cost savings. Both methods meet all USP requirements for the chromatographic system.

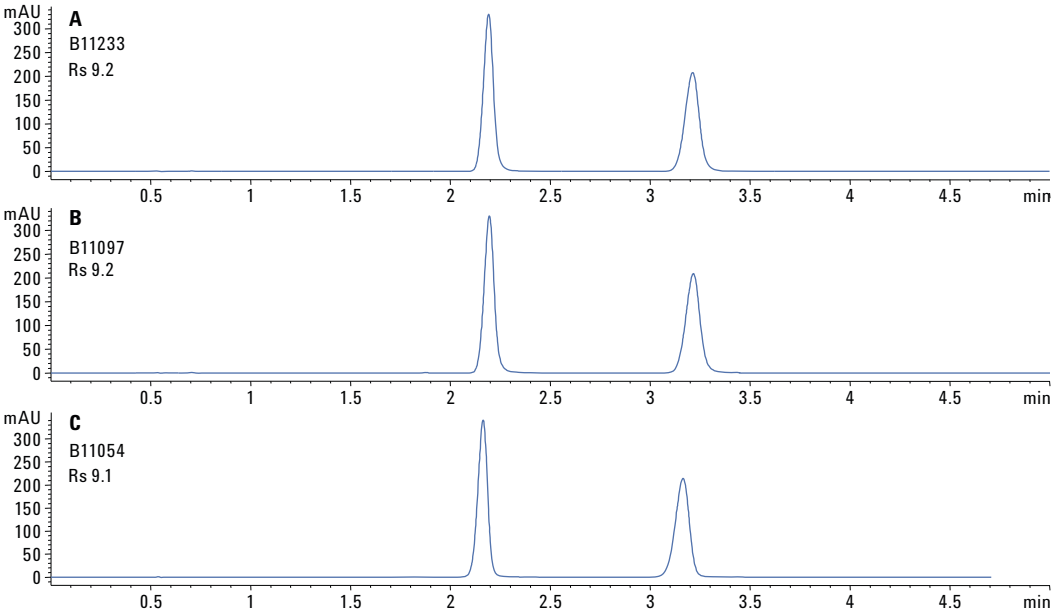


Figure 2. Chromatograms from three different batches of Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm columns.

## References

1. A. Mack "*USP Analysis of Diphenhydramine and Pseudoephedrine Using an Agilent Poroshell 120 EC-CN Column*" Application note, Agilent Technologies, Inc. Publication Number 5991-1687 EN (2013).

2. Anon. Cephradine. USP30-NF25. United States Pharmacopeia, Rockville, MD, USA (2006).

## 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
October 8, 2013
5991-3356EN

Agilent Technologies