Transfer the USP Method for Ceftizoxime from a Traditional 5-µm Column to an Agilent Poroshell 120

Application Note

Small Molecule Pharmaceuticals and Generics

Author

Rongjie Fu

Agilent Technologies (Shanghai) Co. Ltd

Abstract

A method for ceftizoxime was run on a traditional 5-µm column according to the United States Pharmacopeia (USP) assay for this drug. The method was then transferred to a superficially porous Agilent Poroshell 120 column, which allows for significant time and solvent savings within the guidelines of USP Chapter 621. The system requirements were all met with the Poroshell 120 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 analysis of ceftizoxime [2] using a traditional 5-µm column, which is then transferred to shorter 2.7-µm superficially porous Agilent Poroshell 120 columns. The analyses were compared according to the USP chromatographic system requirements.

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Materials and Methods

All reagents and solvents were HPLC or analytical grade. The standards of ceftizoxime and salicylic acid were purchased from USP. Citric acid monohydrate, dibasic sodium phosphate, and acetonitrile were purchased from J&K Scientific Ltd, Beijing.

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

Conditions

Columns:	Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm (p/n 697975-902) Agilent Poroshell 120 EC-C18, 4.6 × 100 mm, 2.7 µm (p/n 695975-902) Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)
Eluent:	Buffer, pH 3.6 (1.42 g citric acid monohydrate and 1.73 g dibasic sodium phosphate in 1,000 mL water):acetonitrile (90:10)
Injection volume:	10 µL for the 4.6 × 250 mm column 4 µL for the 4.6 × 100 mm column 3 µL for the 4.6 × 75 mm column
Flow rate:	2.0 mL/min
Temperature:	25 °C
Detection:	UV, 254 nm

Results and Discussion

Figure 1 shows the system suitability for USP ceftizoxime analysis. The top chromatogram shows the analysis performed as specified by USP with a 4.6 × 250 mm, 5-µm column with L1 packing, which, in this case, was a ZORBAX Eclipse Plus C18. Ceftizoxime and internal standard salicylic acid were easily separated in 12 minutes, and the resolution of the two compounds was 22.6, which is much greater than the USP requirement of 4.0.

The method was then transferred to a 4.6 x 100 mm, 2.7-µm Poroshell 120 EC-C18 column, shown in the middle chromatogram of Figure 1. The analysis was performed in only 4 minutes and the resolution of the two compounds was 25.2, which is a good fit for the USP requirement. The method could be speeded up by using a shorter 4.6 x 70 mm, 2.7-µm Poroshell 120 EC-C18 column, as shown in the bottom chromatogram of Figure 1, which is still allowed under USP Chapter 621.

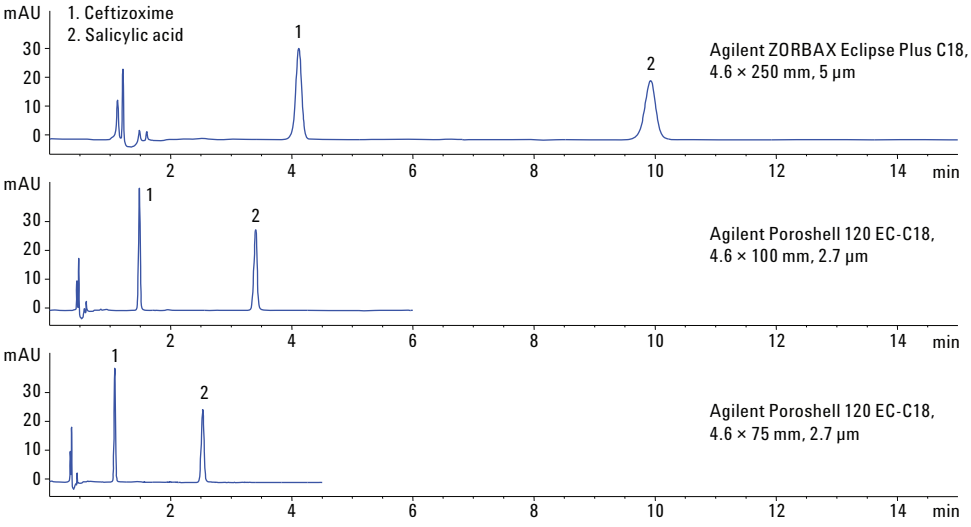

Figure 1. System suitability for USP ceftizoxime assay analysis using Agilent ZORBAX Eclipse Plus C18 and Agilent Poroshell 120 EC-C18 columns.

The USP chromatographic system requirements were all measured according to the USP monograph for ceftizoxime, using all columns. Table 1 lists the USP chromatographic system requirements and measured values on the three columns. The methods on these columns all met the USP chromatographic system requirements.

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

Table 1. USP chromatographic system requirements and measured values for ceftizoxime.

	Agilent column		
USP requirements	**4.6 × 250 mm**	**4.6 × 100 mm**	**4.6 × 75 mm**
The column efficiency determined from the analyte peak is not less than 2,000 theoretical plates.	8,245	12,982	9,834
The tailing factor for the analyte peak is not more than 2.	0.92	1.00	1.06
The resolution (R) between the analyte and internal standard peaks is not less than 4.	22.6	25.2	22.9
The relative standard deviation for replicate injections is not more than 2%.	0.13%	0.12%	0.14%

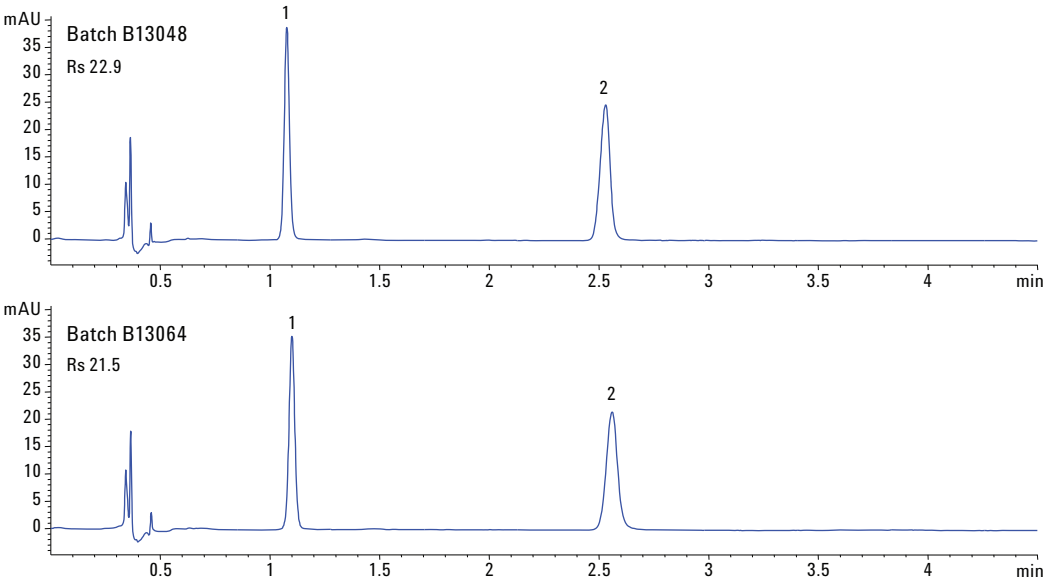

Figure 2. Chromatograms of ceftizoxime and salicylic acid on two different batches of Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm.

Conclusions

The traditional method of USP assay for ceftizoxime 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.

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. Ceftizoxime. USP35-NF30, 2574. United States Pharmacopeia, Rockville, MD, USA (2012).

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Printed in the USA
December 3, 2013
5991-3717EN

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