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

Application Note

Small Molecule Pharmaceuticals and Generics

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Abstract

A method for the analysis of atorvastatin calcium organic impurities was run on a traditional 5-µm column according to the European Pharmacopeia (EP) or United States Pharmacopeia (USP) methods for this drug. The method was transferred to a superficially porous Agilent Poroshell 120 SB-C8 column, which allows for significant time and solvent savings within the guidelines in USP Chapter 621. The system requirements were all met with the Poroshell 120 SB-C8 column.

Agilent Technologies

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 EP/USP organic impurities analysis of atorvastatin calcium [2,3] using a traditional 5-µm USP L7 column, which is then transferred to a shorter 2.7-µm superficially porous Poroshell 120 column. The analyses were compared according to the USP chromatographic system requirements.

Materials and Methods

All reagents and solvents were HPLC or analytical grade. The standards were provided by Menovo Pharmaceutical in China. Tetrahydrofuran, acetonitrile, ammonium acetate and glacial acetic acid were purchased from J&K Scientific Ltd, Beijing.

The HPLC analysis was performed with an Agilent Infinity 1290 Infinity LC System, consisting of:

- Agilent 1290 Infinity Binary Pump (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1290 Infinity Diode Array Detector (G4212A)

Conditions

Column:	Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902) Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm (p/n 697975-302)
Eluent:	A) acetonitrile, stabilizer-free tetrahydrofuran, and buffer (3.9 g/L ammonium acetate in water. Adjust with glacial acetic acid to a pH of 5.0 ± 0.1) (21:12:67) B) acetonitrile, stabilizer-free tetrahydrofuran, and buffer (as above) (61:12:27).

Gradient for the 4.6 × 250 mm column:

Time (min)	%B
0	0
40	0
70	80
85	100
100	100
105	0
115	0

Gradient for the 3.0 × 100 mm column:

Time (min)	%B
0	0
16	0
28	80
34	100
40	100
42	0
46	0

Flow rate:	1.5 mL/min for the 4.6 × 250 mm column, 0.64 mL/min for the 3.0 × 100 mm column
Temperature:	35 °C
Injection volume:	10 µL for the 4.6 × 250 mm column, 1.7 µL for the 3.0 × 100 mm column
Detection:	UV 244 nm

Results and Discussion

Figure 1 shows the system suitability for the analysis of atorvastatin calcium organic impurities. The chromatograms in Figure 1 show the analysis performed as specified by the USP, with a 4.6 × 250 mm, 5-µm column with L7 packing, which in this case was on ZORBAX XDB-C8 and SB-C8 columns. Atorvastatin and four impurities were easily separated by SB-C8 in 115 minutes, and the resolution of the atorvastatin and impurity B was 1.69, which is much greater than the USP requirement of 1.5. However, the XDB-C8 column could not resolve the peak pairs. The non-endcapped phase of SB-C8 provides more unique selectivity than the endcapped phase.

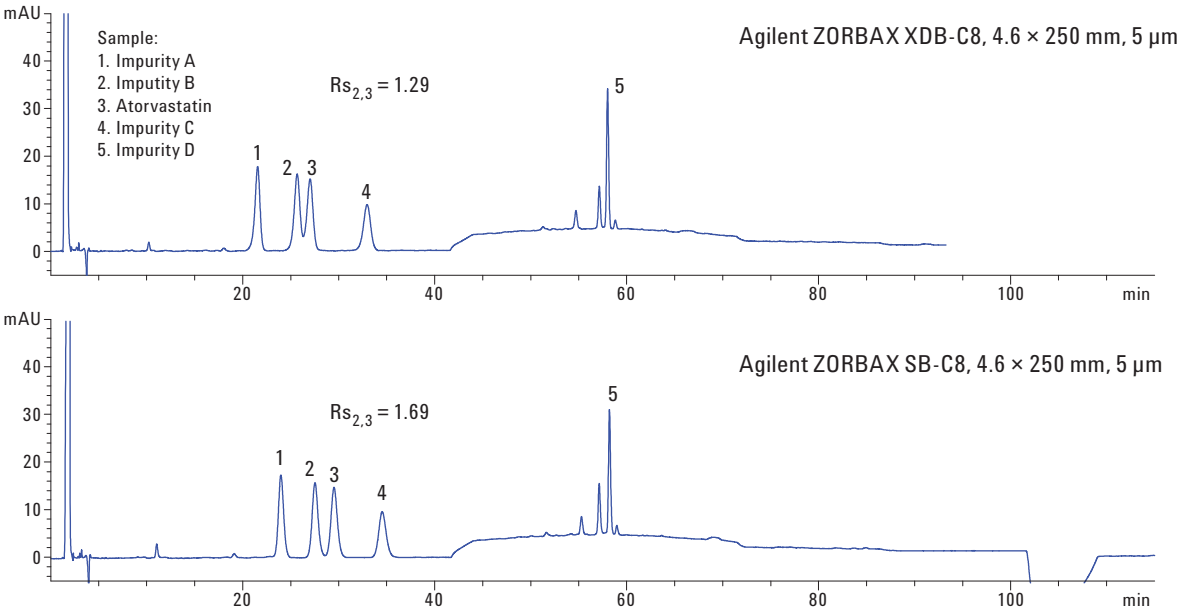

Figure 1. System suitability of USP atorvastatin calcium impurity analysis using Agilent ZORBAX XDB-C8 and Agilent ZORBAXSB-C8 columns.

The method was then transferred to 3.0 × 100 mm, 2.7-µm Poroshell 120 EC-C8 and Poroshell 120 SB-C8 columns, shown in Figure 2. The analysis was performed in 40 minutes, and the resolution of atorvastatin and impurity B on Poroshell 120 EC-C8 was 0.97, but was 1.67 on Poroshell 120 SB-C8, which meets the USP requirement.

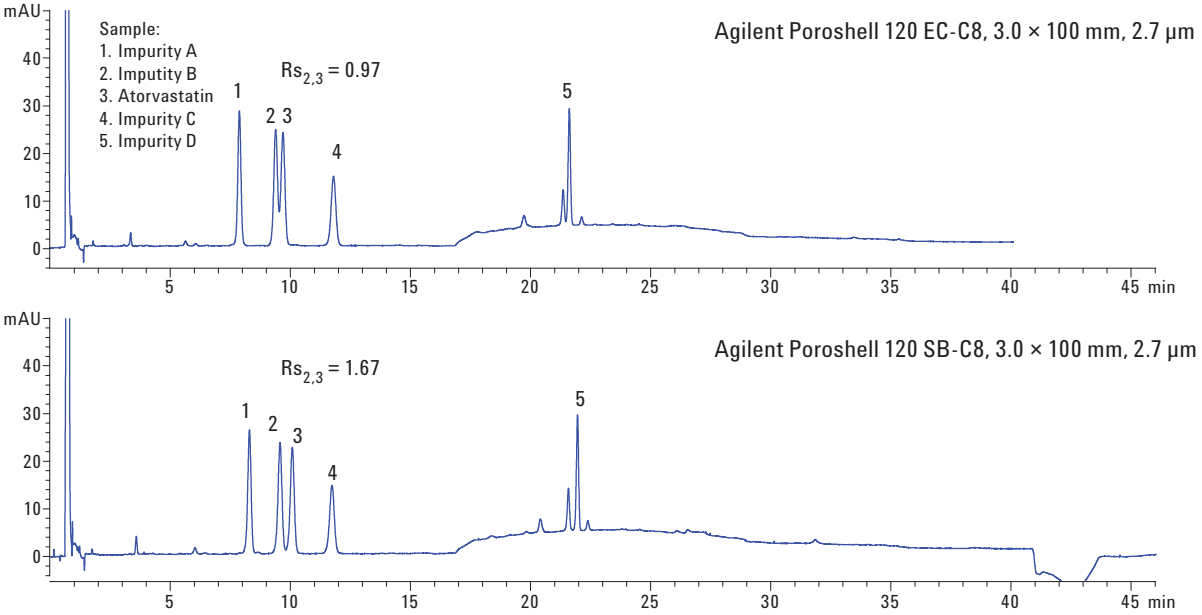

Figure 2. System suitability of USP atorvastatin calcium impurity analysis using Agilent Poroshell 120 EC-C8 and Agilent Poroshell 120 SB-C8 columns.

Using Poroshell 120, all the impurities were separated from atorvastatin, and the analysis time was reduced by 60% (Figure 3).

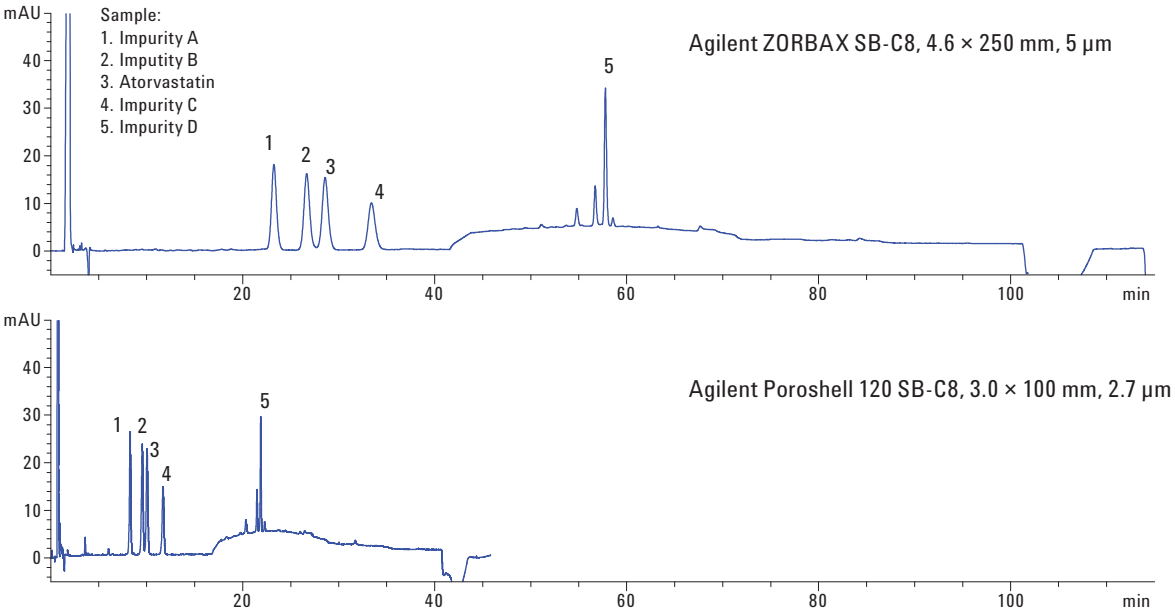

Figure 3. Chromatograms comparing the analysis of USP atorvastatin calcium impurity on Agilent ZORBAX SB-C8 and Agilent Poroshell 120 SB-C8 columns.

The real sample of atorvastatin was analyzed by ZORBAX SB-C8 (Figure 4) and Poroshell 120 SB-C8 (Figure 5). The chromatograms of the sample showed no peaks for the target impurities. Both methods, using a traditional 5-µm column and 2.7-µm Poroshell 120 column, were fit for impurities' analysis in atorvastatin.

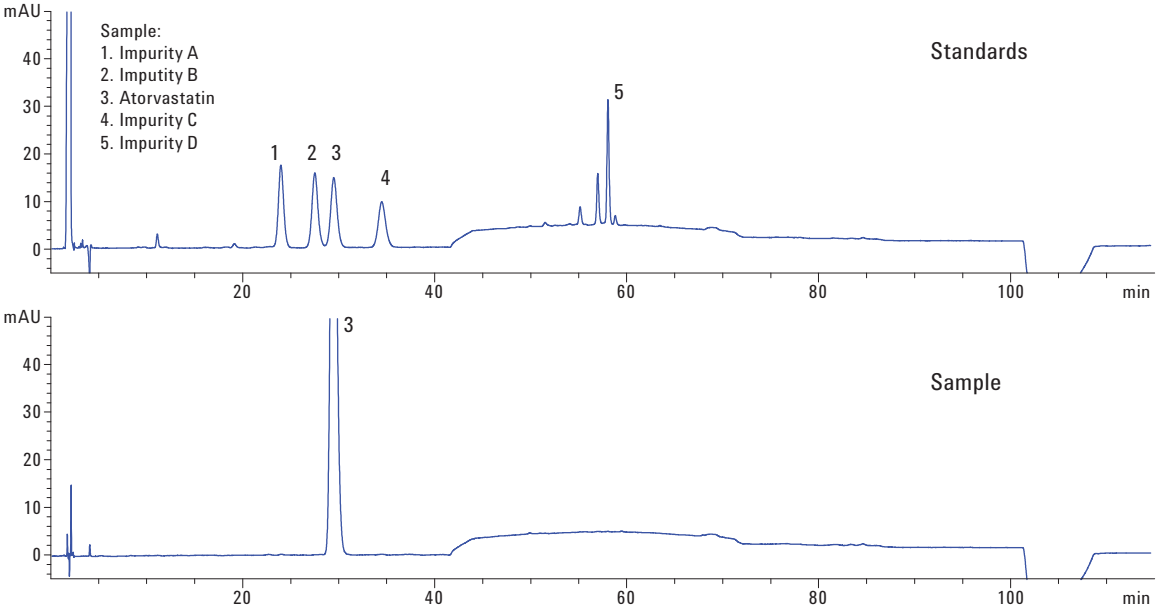

Figure 4. System suitability for atorvastatin analysis demonstrated using standard and real samples with an Agilent ZORBAX SB-C8.

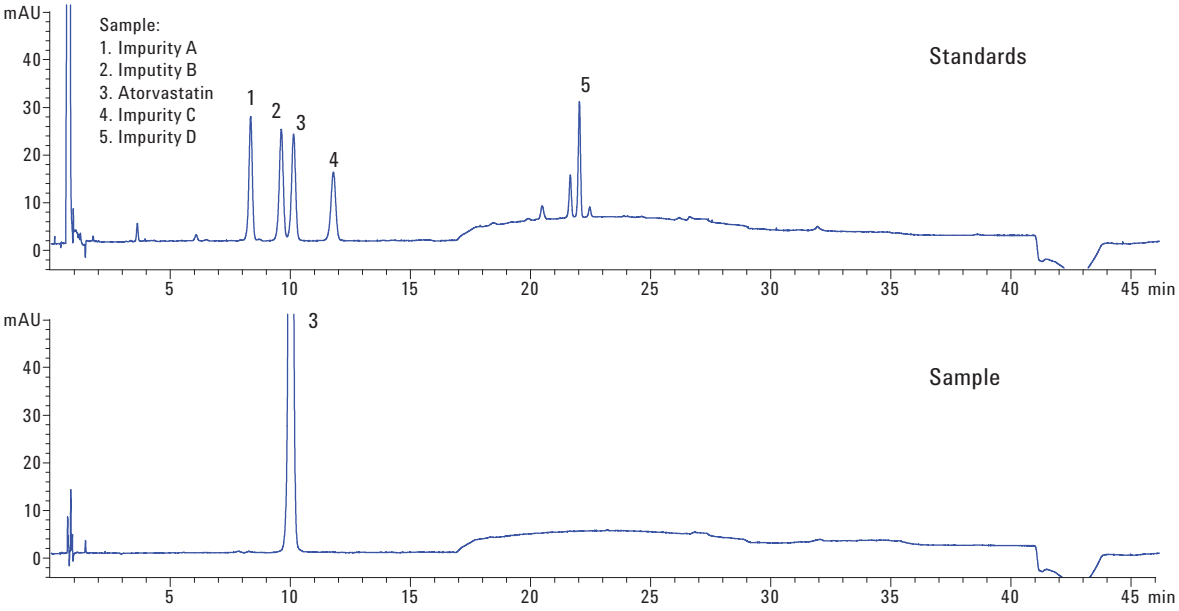

Figure 5. System suitability for atorvastatin analysis demonstrated using standard and real samples with an Agilent Poroshell 120 SB-C8.

The chromatographic system requirements were all measured according to the USP monograph for atorvastatin calcium using both columns. Table 1 shows that measured values on both columns met the USP chromatographic system requirements.

Table 1. The USP chromatographic system requirements and measured values for atorvastatin.

USP Requirements	Agilent ZORBAX SB-C18, 5 µm	Agilent Poroshell 120 SB-C8, 2.7 µm
Resolution: NLT 1.5 between the peaks for atorvastatin impurity B and atorvastatin	1.69	1.67
Tailing factor: NMT 1.6	1.07	0.92
Relative standard deviation: NMT 0.6%	0.11%	0.15%

Conclusions

The traditional method of USP/EP assay for atorvastatin 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, using Agilent ZORBAX SB-C8 and Agilent Poroshell 120 SB-C8, meet all the USP requirements for the chromatographic system.

References

1. Anne 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. *Atorvastatin Calcium*. USP-35 USP. The United States Pharmacopeial Convention, Rockville, MD, USA (2013).
3. Anon. *Atorvastatin Calcium Trihydrate*. EP Monograph, 04/2011:2191 EDQM Council of Europe, Strasbourg, France (2011).

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