Fast Analysis of *Zanthoxylum nitidum* Using Agilent Poroshell 120 EC-18 Columns

Application Note

Traditional Chinese Medicine

Author

Yun Zou
Agilent Technologies (Shanghai) Co. Ltd
412 Ying Lun Road
Waigaoqiao Free Trade Zone
Shanghai 200131, P.R. China

Abstract

The Chinese Pharmacopoeia method for analyzing Zanthoxylum nitidum was reproduced successfully on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column. The good separation was transferred easily to an Agilent Poroshell 120 EC-C18 column; excellent peak shape of nitidine chloride ensured accurate quantitation. Gradient time decreased from 35 minutes to 17.5 minutes on a 75 cm Poroshell 120 column, and to 12 minutes on a 50 cm Poroshell 120 column. Analysis time decreased further, to below 4 minutes by increasing the flow rate; backpressure was still below 400 bar and so the analysis could be run on a traditional HPLC instrument. The Poroshell 120 columns shortened run time while maintaining high resolution of complex traditional Chinese medicine (TCM) components, thereby increasing sample throughput and reducing costs.

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Introduction

Zanthoxylum nitidum (Roxb.) DC., also known as liangmianzhen, is a plant of the Rutaceae family. It is widely distributed throughout the southeastern part of China, and could also be found in India, northern Queensland and Australia [1]. Zanthoxylum nitidum is a commonly used Chinese herbal medicine, which can promote blood circulation, dissipate blood stasis, detumescence, and relieve pain. *Zanthoxylum nitidum* contains many alkaloids, of which nitidine chloride is the major active component that exhibits the main pharmacological and biological activity [2]. Nitidine chloride is best known for its anti-cancer activities [3]. The Chinese Pharmacopoeia [4] specifies that the content of nitidine chloride in Z. nitidum as determined by HPLC shall not be less than 0.13% to control the quality of the medicinal material.

Agilent Poroshell 120 EC-C18, 2.7 µm columns are packed with superficially porous particles, which deliver fast separation and achieve performance similar to sub 2 µm totally porous materials. However, since they use 2-µm column frits like those found on 5 µm particle size columns, they require no additional sample preparation. This allows a seamless method transfer to the Poroshell 120 columns from established methods using 5-µm columns. This makes the Poroshell 120 columns very suitable for the analysis of herbal medicines because these samples are often very complex. The Poroshell 120 columns can be used to reduce the analysis time that is normally quite long on a 5-µm column, while providing the high resolution needed for these samples.

In this work, a gradient method was successfully transferred from a traditional 4.6 × 150 mm, 5 µm column to two different 2.7 µm Poroshell 120 EC-18 columns to increase the speed of the analysis and achieve good resolution and high efficiency, while exceeding the requirements of the Chinese Pharmacopoeia.

Experimental

Reagents and chemicals

Nitidine chloride was obtained from the National Institute for the Control of Pharmaceuticals and Biological Products of China (NICPBP, Beijing, China). Acetonitrile (ACN) and methanol were obtained from Sinopharm Chemical Reagent (Shanghai, China). Water was prepared with a Milli-Q pure water system.

A standard solution of nitidine chloride was prepared by weighing the appropriate standard and dissolving in 70% methanol aqueous solution at 50 µg/mL.

Sample preparation

The raw TCMs of *Z. nitidum* were purchased from a local TCM store. A powdered sample (1 g) was extracted with 20 mL of 70% methanol aqueous solution in an ultrasonic water bath for 30 minutes. Extraction was repeated twice. The extracted solutions were combined and diluted to 50 mL with 70% methanol aqueous solution. This solution was then filtered with a 0.45-µm regenerated cellulose membrane filter (p/n 5064-8221) and injected directly into the HPLC system.

Instrumentation

Analyses were performed on an Agilent 1290 Infinity LC System with a binary pump (G4220A), a thermostatted column compartment (TCC, G1316C), an autosampler (G4226A) and a diode array detector (DAD, G4212A). Table 1 shows method parameters for various column dimensions.

The columns used were:

Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm (p/n 959993-902)

Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm (p/n 697975-902)

Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm (p/n 699975-902)

Table 1. Method Parameters for the Columns

	Agilent Zorbax Eclipse Plus C18 4.6 × 150 mm, 5 µm	**Agilent Poroshell 120 EC-C18 4.6 × 75 mm, 2.7 µm**	**Agilent Poroshell 120 EC-C18 4.6 × 50 mm, 2.7 µm**	
Mobile phase	A: 0.1% formic acid-triethylamine (pH = 4.5), (0.1% (v/v) formic acid aqueous solution adjusted with triethylamine to a pH of 4.5) B: acetonitrile			
Gradient	0 min 20% B 30 min 50% B 35 min 100% B	0 min 20% B 15 min 50% B 17.5 min 100% B	0 min 20% B 10 min 50% B 12 min 100% B	0 min 20% B 2.7 min 27% B 3 min 100% B
Flow rate	1.5 mL/min	1.5 mL/min	1.5 mL/min	3 mL/min
Injection volume	10 µL	5 µL	4 µL	4 µL
TCC temperature	30 °C	30 °C	30 °C	30 °C
Detector	UV 273 nm	UV 273 nm	UV 273 nm	UV 273 nm

Results and Discussion

The original separation method of Z. nitidum published in the 2010 Chinese Pharmacopoeia was repeated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column. Figure 1 shows the chromatograms of nitidine chloride standard solution and *Z. nitidum* extract. Due to the complicated matrix, this type of separation typically requires more than 35 minutes. Nitidine chloride can be baseline separated with excellent peak shape. Performance of the column was shown in Figure 2A.

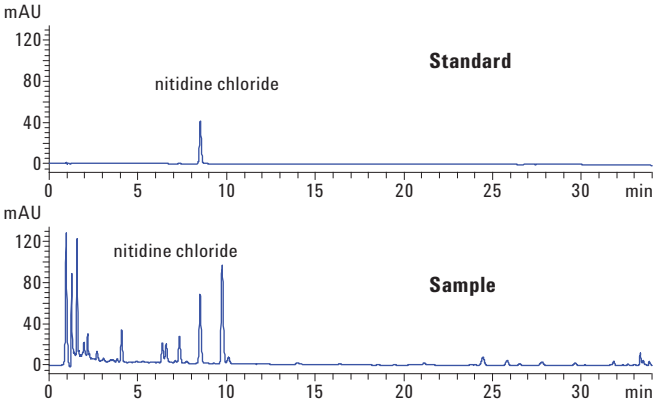

Figure 1. Original method for analyzing Zanthoxylum nitidum on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column.

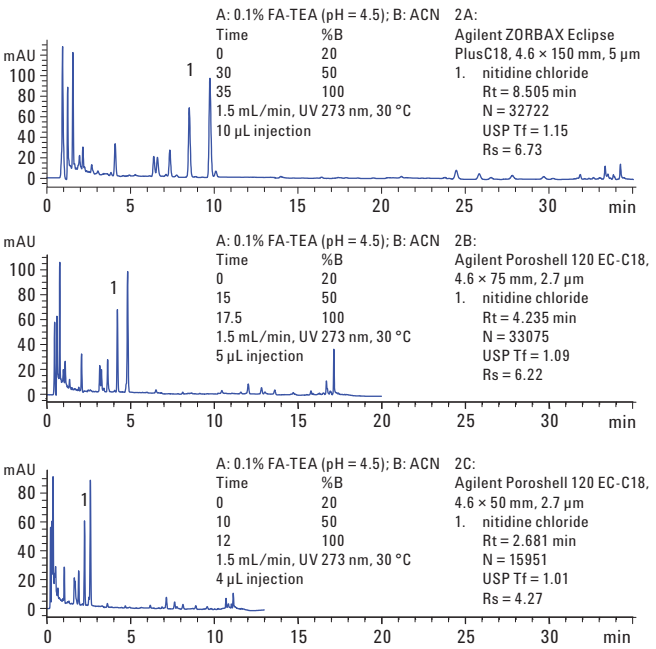

Figure 2. Overlaid chromatograms of Zanthoxylum nitidum using Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm; Agilent Poroshell 20 EC-C18, 4.6 × 75 mm, 2.7 µm and Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column.

The original method was scaled from a 4.6×150mm, 5 µm column to an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm column. The flow rate should not be changed, according to Equation 1, to maintain the same linear velocity.

Eq 1

$$F_1/(r_1)^2 = F_2/(r_2)^2$$

where

F_1 is the flow rate of original column
F_2 is the flow rate of new column
r_1 is the radius of original column
r_2 is the radius of new column

The gradient time is proportional to the column length while maintaining the original separation. Since the original column is 4.6 × 150 mm, using a 4.6 × 75 mm column at the same linear velocity shortens the gradient time by 75/150 or a factor of 2, so the injection volume is decreased by half to avoid sample overload. The analysis time also decreased by a factor of 2, from 35 minutes to 17.5 minutes, as shown in Figure 2A and 2B. However, compared to the original method, the chromatogram showed equivalent or slightly better peak shape, theoretical plates and resolution with the Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm column. The backpressure of this Poroshell column analysis is less than 250 bar making it possible to use this faster method on any HPLC or UHPLC.

Similarly, the analysis time could be further shortened by using a shorter column. As shown in Figure 2C, the new method saved two-thirds of the original analysis time and the backpressure of 176 bar was quite acceptable for a 400-bar HPLC when using a Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column. The value for theoretical plates of nitidine chloride greatly exceeded the requirement of the 2010 Chinese Pharmacopoeia (N>2500).

This method could be further optimized based on the performance of the Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column. Firstly, the cycle time could be further reduced by using twice the flow rate, because the Van Deemter curve of the superficially porous Poroshell 2.7 µm particles is similar to columns with 1.8 µm particles. Therefore, the efficiency of the Poroshell 120 column does not decrease significantly at higher flow rates. Nitidine chloride is the target compound in this method to control the quality of *Z. nitidum*. After nitidine chloride elutes out of the detector, the proportion of ACN in the mobile phase could be increased to wash the contaminants in *Z. nitidum* out of the column quickly.

As illustrated in Figure 3, having optimized the flow rate and gradient slope for the best possible balance between resolution and analysis time, the analysis time fell to approximately one-tenth of the original analysis time with some loss in resolution and theoretical plates, which were much better than the regulation requirements. The backpressure was less than 370 bar. This pressure was fine for the column, which could be used up to 600 bar. Although a traditional 400-bar HPLC instrument could be used for this method, it is better to use an HPLC or UHPLC with a pressure limit of at least 600 bar.

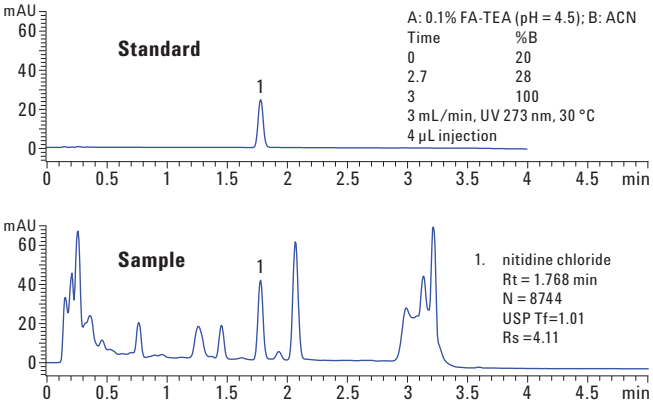

Figure 3. Optimized method for analyzing Zanthoxylum nitidum on an Agilent Poroshell 120 EC-C18, 4.6 × 50 mm, 2.7 µm column.

Conclusions

A method for the analysis of nitidine chloride in *Z. nitidum* was successfully transferred from a traditional 4.6 × 150 mm, 5 µm column to an Agilent Poroshell 120 column. The Poroshell 120 2.7 µm particle columns provide high efficiency and high resolution allowing for faster methods. This is similar to the performance that could be achieved with sub 2 µm columns. But the Poroshell 120 column operates at a lower pressure such that a 400-bar instrument can run the Poroshell 120 column method for routine analysis. A higher flow rate reduces analysis time to less than 4 minutes. The transferred methods are fast with high efficiency, providing good separation and peak shape, and therefore provide substantial time and cost savings.

References

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