



# Analysis of Saponins in Notoginseng using Agilent Poroshell 120, 4 $\mu\text{m}$ Columns

## Application Note

Small Molecule Pharmaceuticals

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### Abstract

A regulated method in the China Pharmacopoeia (CHP) [1] for the analysis of saponins in notoginseng was run using a 4  $\mu\text{m}$  superficially porous particle column. Four target compounds were well separated, and the system requirements were all met with the Agilent Poroshell 120 EC-C18, 4  $\mu\text{m}$  column. The results were compared to analysis on 3.5 and 5  $\mu\text{m}$  totally porous particle columns, and a 2.7  $\mu\text{m}$  Poroshell 120 column. The 4  $\mu\text{m}$  Poroshell 120 column has more advantages over totally porous particle columns. In addition, the method was transferred to a short column of 100 mm, saving more than 30% in analysis time and solvents, and even more when run at a higher flow rate, while still at a lower backpressure.

### Introduction

Notoginseng is a herb that has been used in China extensively since the end of the 19th century. It has acquired a very favorable reputation for treatment of blood disorders, including blood stasis, bleeding, and blood deficiency. Notoginseng and ginseng have many similar components, as they belong to the same genus, *Panax*. Important components of notoginseng are saponins, favonosides, polysaccharides, and amino acids. Notoginseng contains high levels of Rb1, Rd, and Rg1 ginsenosides. The Rb1, Rd, and Rg1 content of notoginseng was higher than that of ginseng in one study [2]. The China Pharmacopoeia (CHP) requires determination of R1, Rg1, and Rb1 using an HPLC method with analysis time over 60 minutes [1].



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Superficially porous 4  $\mu\text{m}$  particles have high efficiency, similar to that of 3.5  $\mu\text{m}$  totally porous particles and twice the efficiency of 5  $\mu\text{m}$  totally porous particles. In this application note, the original CHP method was run on an Agilent ZORBAX Eclipse Plus C18 column with 5  $\mu\text{m}$  totally porous particles, and then replaced with an Agilent Poroshell 120 EC-C18 column with 4  $\mu\text{m}$  superficially porous particles, without any method adjustment. The method was also transferred to shorter columns to save time and reduce solvent use. Figure 1 shows the saponins investigated in this work.

## Materials and methods

All reagents and solvents were HPLC or analytical grade. The notoginseng sample was provided by a local pharmaceutical company in China. Acetonitrile was purchased from J&K Scientific Ltd, Beijing. The HPLC analysis was performed with an Agilent 1290 Infinity LC System with:

- 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

Columns: Agilent Poroshell 120 EC-C18 4.6  $\times$  100 mm, 4  $\mu\text{m}$  (p/n 695970-902),  
 Agilent Poroshell 120 EC-C18, 4.6  $\times$  150 mm, 4  $\mu\text{m}$  (p/n 693970-902),  
 Agilent Poroshell 120 EC-C18, 4.6  $\times$  100 mm, 2.7  $\mu\text{m}$  (p/n 695975-902),  
 Agilent ZORBAX Eclipse Plus C18, 4.6  $\times$  100 mm, 3.5  $\mu\text{m}$  (p/n 959961-902),  
 Agilent ZORBAX Eclipse Plus C18, 4.6  $\times$  150 mm, 5  $\mu\text{m}$  (p/n 959993-902)

Mobile phase: A) Water  
 B) Acetonitrile

Gradient for 4.6  $\times$  150 mm columns:

| Time (min) | %A |
|------------|----|
| 0          | 81 |
| 12         | 81 |
| 60         | 64 |
| 61         | 10 |
| 65         | 10 |
| 66         | 81 |
| 70         | 81 |

Gradient for 4.6  $\times$  100 mm columns:

| Time (min) | %A |
|------------|----|
| 0          | 81 |
| 8          | 81 |
| 40         | 64 |
| 40.5       | 10 |
| 43.5       | 10 |
| 44         | 81 |
| 47         | 81 |

Temperature: 30  $^{\circ}\text{C}$

Flow rate: 1.0 mL/min

Injection volume: 10  $\mu\text{L}$  for 150 mm columns; 6.7  $\mu\text{L}$  for 100 mm columns

Detection: UV, 203 nm

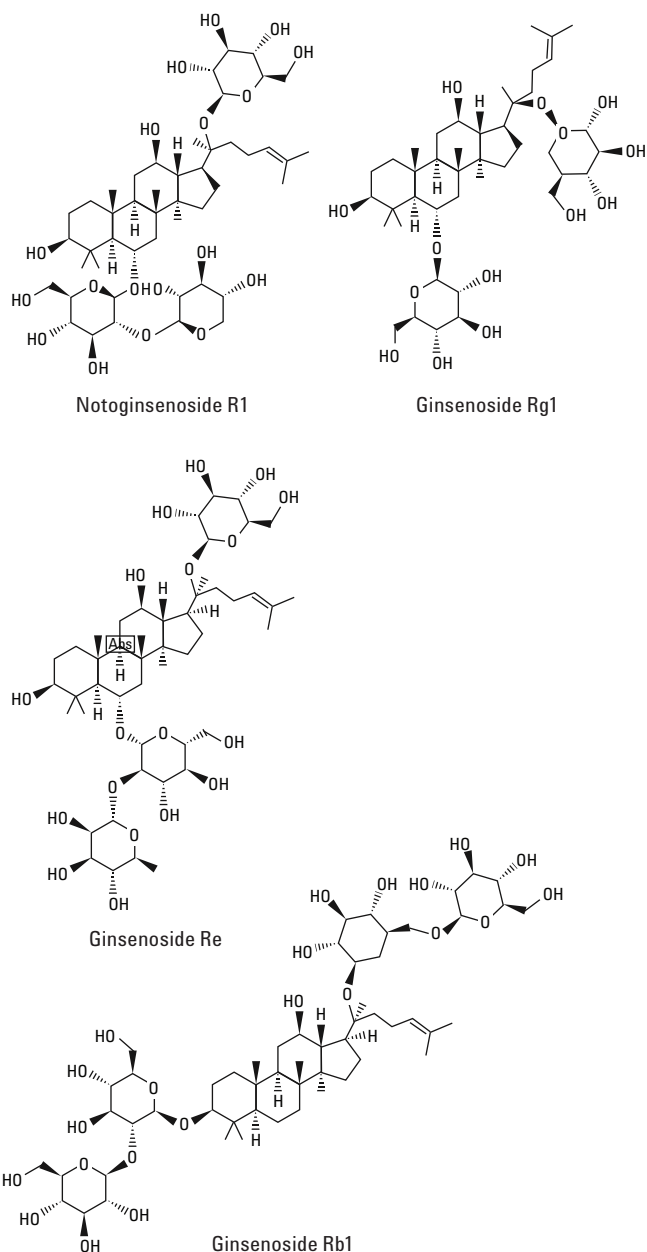


Figure 1. Structures of saponins separated in this study.

## Results and Discussion

The original method regulated by CHP to separate R1, Rg1, Re, and Rb1 was repeated on a ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm column. The resolution of compound pairs of ginsenoside Rg1 and Re was over 1.5 (lower chromatogram in Figure 2). The method was then run on the Poroshell 120, 4 μm column with the same configuration, and the resolution increased significantly from 1.5 to 2.3 (upper chromatogram in Figure 2). The peak width dropped by 50% compared to the 5 μm column. Although the backpressure increased by 50%, the pressure of the 4 μm column was still acceptable for most instruments.

The original method was then transferred to shorter 4.6 × 100 mm columns with different particle sizes. The gradient time was proportional to the column length while maintaining the original separation. The injection volume was decreased proportionately to avoid sample overload. The analysis time, therefore, decreased from 60 to 40 minutes, as shown in Figure 3.

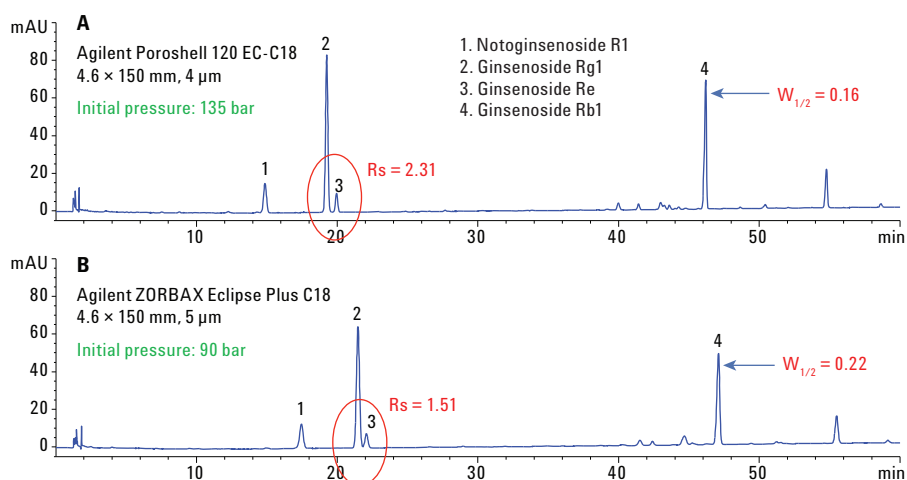


Figure 2. Original China Pharmacopoeia method for analyzing notoginseng on Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 μm and Agilent Poroshell 120 EC-C18, 4 μm columns.

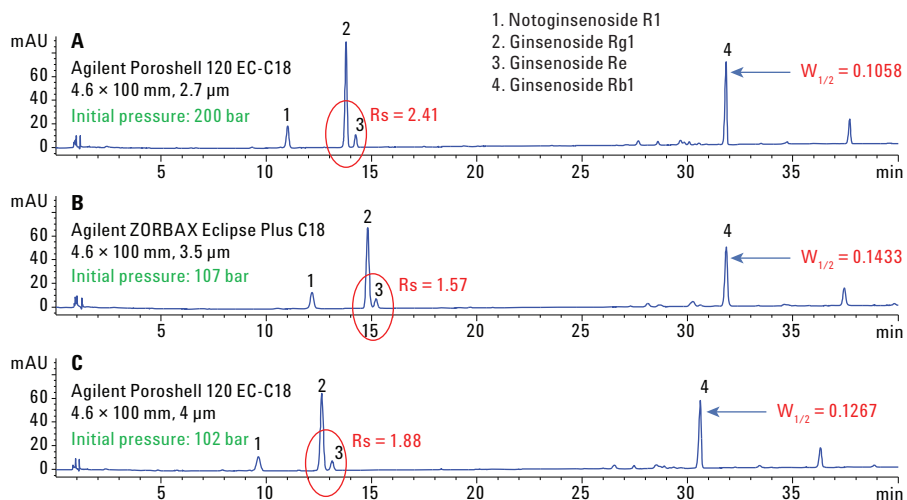


Figure 3. Chromatograms on shorter columns with different particle sizes.

The 4  $\mu\text{m}$  column had similar efficiency to the column with 3.5  $\mu\text{m}$  totally porous particles, and gave increased resolution of difficult pairs, but with decreased retention. Though the 4  $\mu\text{m}$  column provided much lower efficiency and resolution than the 2.7  $\mu\text{m}$  Poroshell 120 column, it generated only half the backpressure. Thus, the 4  $\mu\text{m}$  Poroshell 120 column can be used on instruments with very low pressure limits, while benefiting from increased resolution and efficiency.

The separation time was reduced with a short column, and the analysis could be even faster when the flow rate was increased with some compromise of resolution and performance, which is still acceptable for quantitative measurement (Figure 4).

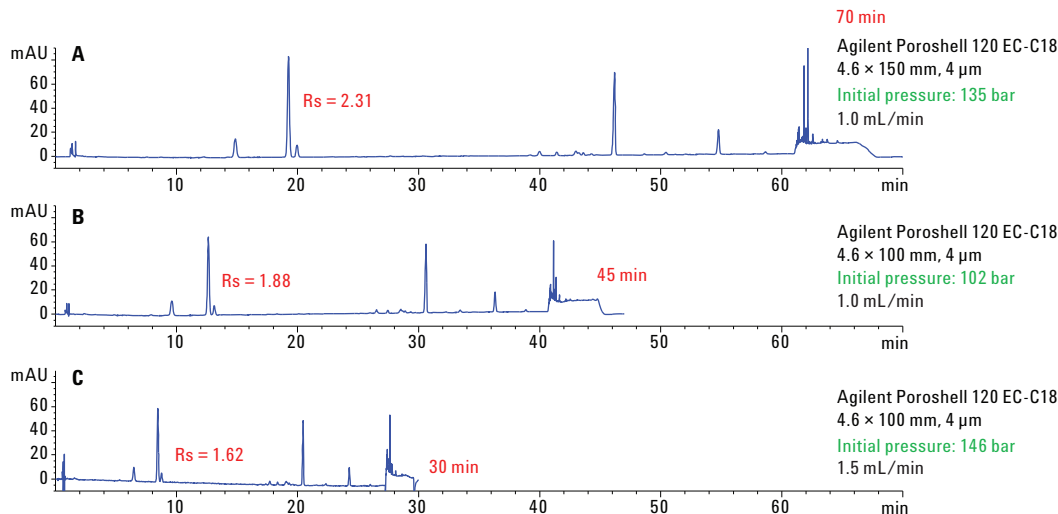


Figure 4. Overlay chromatograms of notoginseng analysis with different lengths and flow rates.

## Conclusions

The China Pharmacopoeia method for the analysis of notoginseng was successfully achieved with both a 4.6 × 150 mm, 5  $\mu\text{m}$  column and an Agilent Poroshell 120, 4  $\mu\text{m}$  column. The 4  $\mu\text{m}$  column could easily replace the 5  $\mu\text{m}$  column without any method change. A shorter column could be used for quick analysis while maintaining resolution and efficiency. Due to the low pressure, a 200-bar instrument can run this method.

## References

1. Notoginseng, *China Pharmacopoeia*, edition **2015**.
2. Zhu, S.; Zou, K.; Fushimi, H.; Cai, S.; Komatsu, K. Comparative study on triterpene saponins of ginseng drugs. *Planta Medica* **2004**, *70*, 666-677.

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