# Analysis of Danshen (Salvia Miltrorrhiza) and Compound Danshen Dropping Pills using Poroshell 120 Superficially Porous LC Columns

Application

Pharmaceuticals


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## Abstract

A popular traditional Chinese medicine, Danshen (Salvia Miltrorrhiza), and its preparation Compound Danshen Dropping Pills were analyzed by HPLC with Agilent Poroshell 120 LC columns. The columns are made from superficially porous particles that have a solid core (1.7 µm) and porous outer layer with a 0.5-µm diffusion path. The advantages of the new column type include high performance similar to that of sub-2-µm particles but with 40-50% lower back pressure, and high peak capacity. The method on the Poroshell 120 column was compared to that generated on a UPLC with a Waters HSS T3 column. The Poroshell 120 column provided excellent peak shape and was operated at a pressure of less than 287 bar. The method can be run on any HPLC.


## Introduction

Danshen (Salvia miltiorrhizae) is widely used as a traditional Chinese medicine (TCM), often in combination with other herbs. Remedies containing Danshen are used traditionally to treat a variety of ailments, particularly cardiac (heart) and vascular (blood vessel) disorders such as atherosclerosis ("hardening" of the arteries with cholesterol plaques) or blood clotting abnormalities. Danshen Dropping Pills are a compound preparation consisting of Danshen, notoginseng, borneol, and other compounds and are used to treat for coronary heart disease and angina.[1]

Because there are so many compounds in the matrix, quality control for TCM's and other natural products is very difficult. HPLC methods can be used for quantitive analysis of only one or several compounds in the TCM, which may not represent the real quality of the TCM. The current trend is to find a unique chromatogram to be used like a fingerprint for a respective material. The more peaks found, the more information is presented for the sample. So the peak capacity and resolution are important for the TCM fingerprint to control its quality.

Regulations have included the Waters UPLC method for TCM fingerprint analysis [2]. In this note, we developed methods with an Agilent Poroshell 120 that can be run on any LC instrument.

## Experimental

### Sample preparation

Pill preparation: Dissolve 10 pills in 10 mL of water. Filter with a 0.45 µm Regenerated Cellulose Membrane filter (p/n: 5064-8221) before injecting into the HPLC for analysis.

Danshen preparation: Weigh 2.0 g of Danshen powder, extract with water at 80 °C for 1 hour, filter and bring the aqueous volume to 50 mL. Filter with a 0.45 µm Regenerated Cellulose Membrane filter (p/n 5064-8221) before injecting into the HPLC for analysis.

### HPLC conditions

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

*Table 1. Conditions for Danshen Dropping Pills*

| | | | | | | |
|---|---|---|---|---|---|---|
| Mobile Phase: | A, 0.02% $H_3PO_4$; B, 0.02% $H_3PO_4$ in ACN/water (80/20) | | | | | |
| Gradient: | | | | | | |
| Time (min) | 0 | 1.6 | 1.8 | 8.0 | 8.4 | 10 |
| %B | 9 | 22 | 26 | 39 | 9 | 9 |
| Column | Waters HSS T3, 2.1 × 100 mm, 1.8 µm<br>Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm<br>Agilent Poroshell 120 SB-C18, 4.6 × 100 mm, 2.7 µm | | | | | |
| Column temp | 40 °C | | | | | |
| Flow Rate | 0.4 ml/min for 2.1 mm ID, 0.8 mL/min for 3.0 mm ID, 2mL/min for 4.6 mm ID | | | | | |
| Wavelength | 280 nm | | | | | |
| Injection Volume | 2 µL on 2.1 and 3.0 mm ID columns, 4.0 µL on 4.6 mm ID column | | | | | |

*Table 2. Conditions for Danshen Water Extraction*

| | | | | | | |
|---|---|---|---|---|---|---|
| Mobile Phase | A, 0.02% $H_3PO_4$ :B, ACN | | | | | |
| Gradient | Flow rate=1 ml/min | | | | | |
| Time(min) | 0 | 6 | 14 | 18 | 22 | 28 |
| %B | 10 | 20 | 25 | 30 | 90 | 90 |
| Gradient | Flow rate = 2 ml/min | | | | | |
| Time(min) | 0 | 3 | 7 | 9 | 11 | 14 |
| B% | 10 | 20 | 25 | 30 | 90 | 90 |
| Gradient | Flow rate = 3 ml/min | | | | | |
| Time(min) | 0 | 2 | 4.67 | 6 | 7.33 | 9.33 |
| B% | 10 | 20 | 25 | 30 | 90 | 90 |
| Column | Agilent Poroshell 120 EC-C18, 4.6 × 100mm, 2.7 µm;<br>Agilent ZORBAX RRHT Eclipse Plus C18,<br>4.6 × 100 mm, 1.8 µm | | | | | |
| Column temp | 30 °C | | | | | |
| Wavelength | 280 nm | | | | | |
| Injection Volume | 4 µL | | | | | |

## Results and Discussion

The Compound Danshen Dropping Pill was analyzed on both a Poroshell 120 EC-18 and a Poroshell 120 SB-C18 column, as shown in Figure 1. All eight target compounds were separated on both columns. The Poroshell 120 EC-C18 column provided a little more retention than the Poroshell 120 SB-C18, and the selectivity was different between the two columns. The selectivity difference indicates that the Poroshell 120 EC-C18 is the better choice for this sample because a small impurity peak was separated from peak 6 on the Poroshell 120 EC-C18 but not on the Poroshell 120 SB-C18.

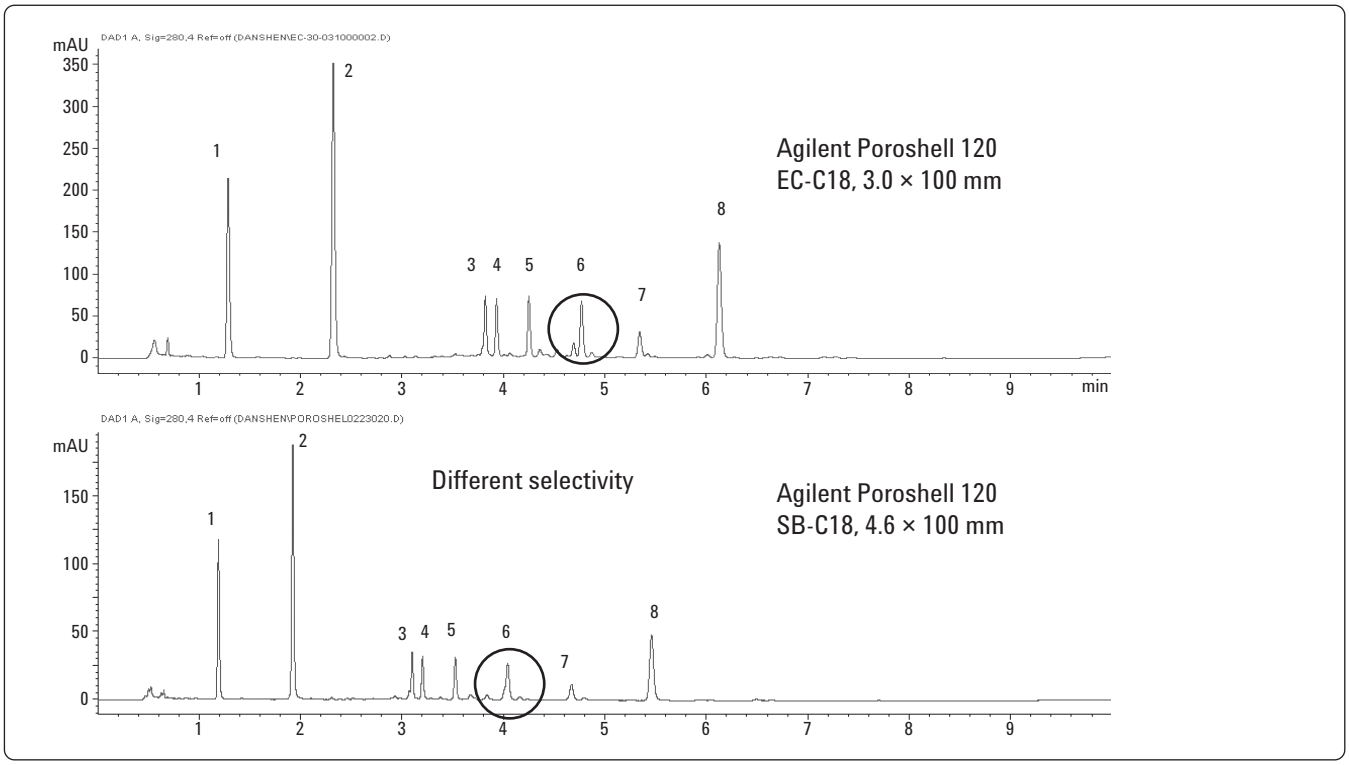


*Figure 1. Danshen Dropping Pills on Agilent Poroshell 120 EC-C18 and SB-C18 columns. Both columns have a particle size of 2.7 µm.*

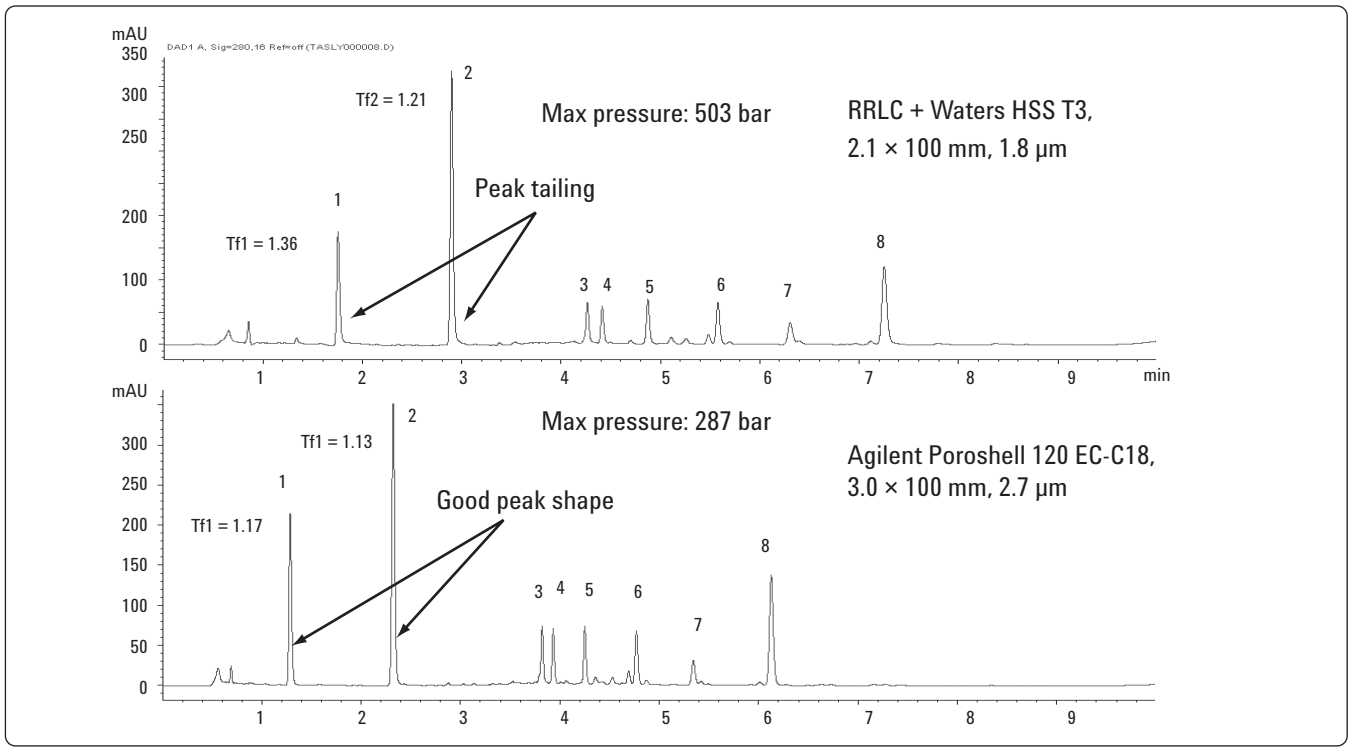


*Figure 2. Comparison of the Agilent Poroshell 120 EC-C18 and competitor column shows the better peak shape and shorter analysis time on the Poroshell 120 column.*

The original method was developed on a Waters HSS T3, 1.8 µm column and was repeated on an Agilent LC with the results shown in Figure 2. A comparison of the results on the Poroshell 120 EC-C18 column and the Waters HSS T3 showed that the Poroshell 120 EC-C18 column had several advantages for this separation. The analysis time was shorter and the peak shape was better than with the Waters HSS T3 column and the resolution was very similar for the eight target compounds. The most important advantage for chromatographers without a UHPLC was that the maximum pressure of the Poroshell 120 EC-C18 column was only 57% of the Waters HSS TS, 1.8 µm column. The method with the Poroshell 120 EC-C18 column can be run on any LC but a high pressure LC is needed to run the same method with the Waters HSS T3 column.

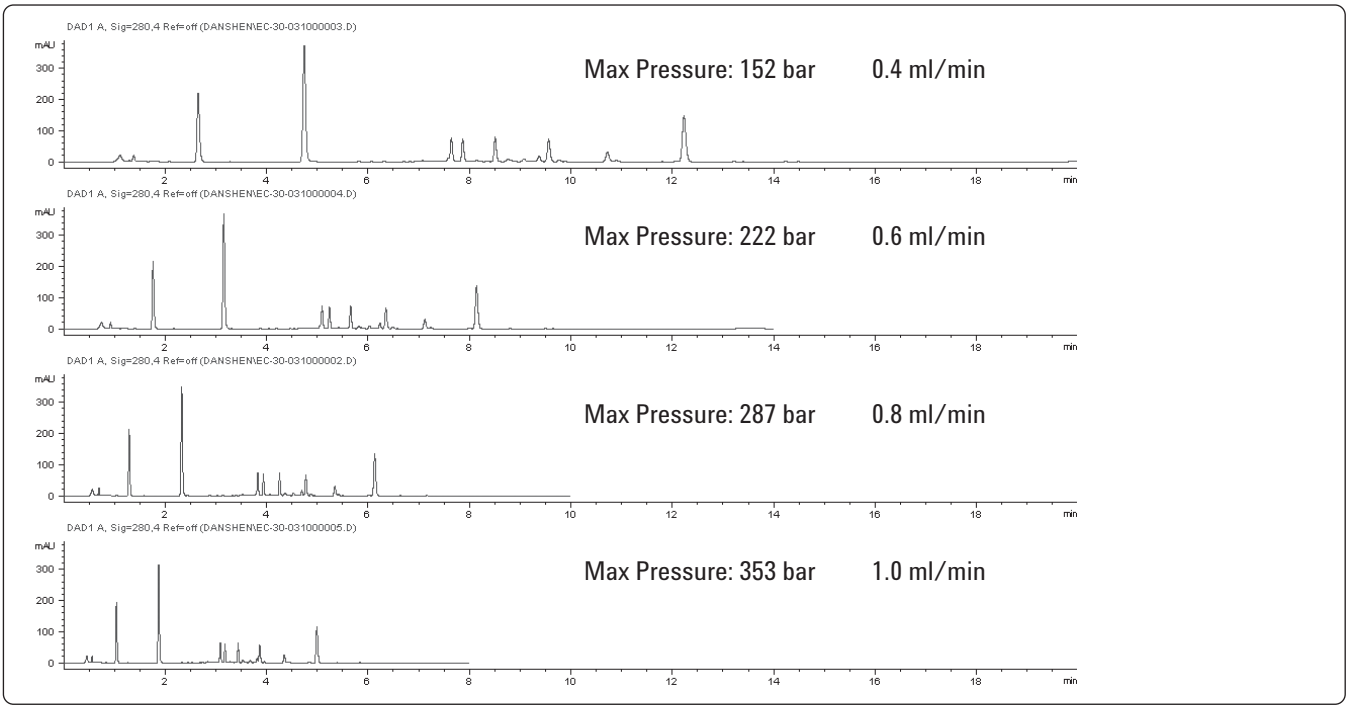


*Figure 3. Chromatograms at different flow rates on the Agilent Poroshell 120 EC-C18, 3.0 × 100 mm, 2.7 µm column.*

Figure 3 shows chromatograms at different flow rates. The separation can perform faster at higher flow rates with almost no loss of resolution, while maintaining pressures under 400 bar.

Water extractions of Danshen were analyzed on the Agilent Poroshell 120 EC-C18 (2.7 µm) and the Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18 (1.8 µm) columns as shown in Figure 4. The Poroshell 120 EC-C18 column with superficially porous particles provided a little less retention and better resolution than the column with sub-2-µm totally porous particles. The small, superficially porous particles generate high efficiency, similar to the efficiency of sub-2-µm columns. The different bonded phases produce results with higher performance and resolution. A small peak was separated from the main peak on the Poroshell 120 EC-C18 column due to these selectivity differences. In addition, the separation can be performed faster at a higher flow rate without resolution loss with this column due to the lower back pressure of the superficially porous column. Columns with superficially porous particles generate higher efficiency and resolution compared to those with totally porous particles due to faster mass transfer in the porous shell particles and the 20% narrower particle size distribution.

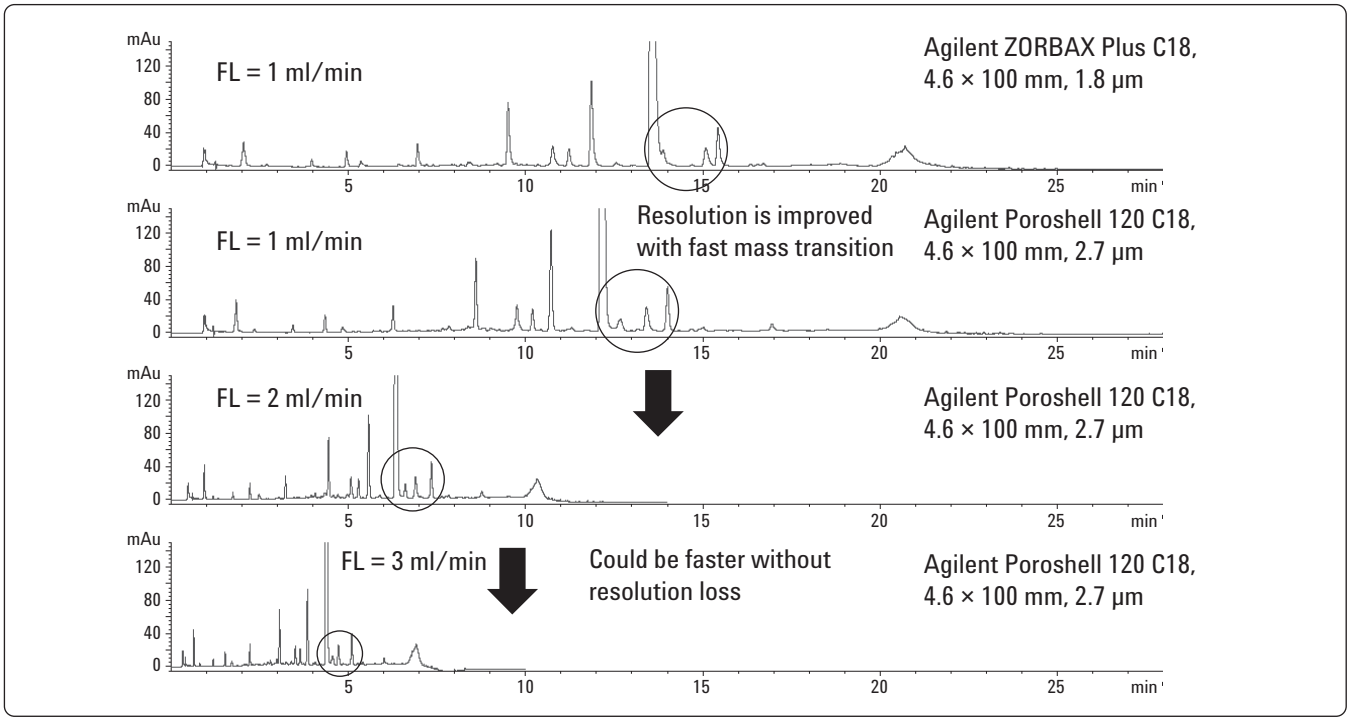


*Figure 4. Water extractions of Danshen on the Agilent Poroshell 120 EC-C18 (2.7 µm) and the Agilent ZORBAX Rapid Resolution HT Eclipse Plus C18 (1.8 µm) column.*

## Conclusion

The superficially porous particles in the Agilent Poroshell 120 columns provide high performance and fast analyses at HPLC pressures. These columns are suitable for the complex sample analysis of a TCM, such as the Danshen extract, because of the high resolution achieved. The pressure on the Agilent Poroshell 120 column is about 40-50% less than that with a column with totally porous sub-2-µm particles. This makes the Agilent Poroshell 120 columns suitable for any LC instrument, especially a standard one with a maximum pressure of 400 bar. Users with standard LC instruments can achieve fast analyses using Poroshell 120 columns.

## Reference

1. http://www.nlm.nih.gov/medlineplus/druginfo/natural/patient-danshen.html
2. Compound Danshen Dropping Pills, China Pharmacopoeia 2010: 906

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