Fingerprint Profiling of Extracts from Danshen (*Salvia miltiorrhiza*) Using Sub-2 µm Superficially Porous LC Columns

Application Note

Pharmaceutical

Author

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Abstract

The extracts of hydrophobic and hydrophilic components in Danshen (*Salvia miltiorrhiza*) are analyzed by UHPLC using sub-2 µm superficially porous LC columns. The method using an Agilent InfinityLab Poroshell 120 was transferred from the regulated methods for fingerprint profiling of Salvia Total Phenolic Acids and Tanshinones in China Pharmacopeia (CHP). The InfinityLab Poroshell 120, 1.9 µm column provided extremely high peak capacity in a shortened analysis time, and is suitable for the analysis of complex samples.

Introduction

Danshen, the dried root of *Salvia miltiorrhiza*, is a traditional Chinese medicine (TCM) used to promote blood flow and treat vascular disease [1]. Because there are so many compounds in the TCM matrix, it is difficult to effectively evaluate the quality and authenticity of a Chinese medicine based on any single active ingredient. To comprehensively evaluate the quality of TCM, the current trend is to find a unique chromatogram to be used as a fingerprint for a respective material. HPLC or UHPLC fingerprinting is the most popular technology used for TCM quality control; using this method, the more peaks that are found, the more information is presented for the sample. Therefore, peak capacity and resolution are important factors for the use of the TCM fingerprint in quality control.

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Danshen is one of the samples regulated in CHP 2015 using HPLC fingerprint profiling for its quality control. Many components with active pharmaceutical effects have been isolated from Danshen, of which salvianolic acids and tanshinones are the most important. Extracts of both total phenolic acids and of tanshinones are regulated by HPLC fingerprint profiling [2].

In this application note, we transferred the CHP methods for total phenolic acids and tanshinones to the newly developed 1.9 µm Agilent InfinityLab Poroshell 120 LC columns.

Materials and Methods

All reagents and solvents were HPLC or analytical grade. Acetonitrile and phosphoric acid were purchased from J&K Scientific Ltd, Beijing. The extracts of Salvia Total Phenolic Acids and Tanshinones and reference standards were provided by a local pharmaceutical company in China. The Salvia Total Phenolic Acids extract was dissolved in water to achieve a concentration of 1 mg/mL, and the tanshinones extract was dissolved in methanol to achieve a concentration of 1 mg/mL.

UHPLC analysis was performed with an Agilent Infinity 1290 LC system including:

- 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)

Columns

- Agilent InfinityLab Poroshell 120 EC-C18, 2.1 mm × 100 mm, 1.9 µm (p/n 695675-902)
- Agilent InfinityLab Poroshell 120 EC-C18, 2.1 mm × 150 mm, 1.9 µm (p/n 693675-902)

UHPLC Conditions for tanshinones

Parameter	Value		
Mobile phase	A) Water (0.026 % phosphoric acid) B) Acetonitrile		
Gradient for	Time (min)	%A	%B
2.1 × 100 mm column	0	80	20
	4	40	60
	10	20	80
Stop time	13 minutes		
Post run	2 minutes		
Gradient for	Time (min)	%A	%B
2.1 × 150 mm column	0	80	20
	6	40	60
	15	20	80
Stop time	20 minutes		
Post run	3 minutes		
Temperature	25 °C		
Flow rate	0.33 mL/min		
Injection volume	1 µL for 100 mm column 1.5 µL for 150 mm column		
Detection	UV 270 nm		

UHPLC Conditions for salvia total phenolic acids

Parameter	Value		
Mobile phase	A) Water (0.05 % phosphoric acid) B) Acetonitrile		
Gradient for	Time (min)	%A	%B
2.1 × 100 mm column	0	90	10
	3	80	20
	7	75	25
	9	70	30
	11	10	90
	14	10	90
Stop time	14 minutes		
Post run	2 minutes		
Gradient for	Time (min)	%A	%B
2.1 × 150 mm column	0	90	10
	4.5	80	20
	10.5	75	25
	13.5	70	30
	16.5	10	90
	21	10	90
Stop time	21 minutes		
Post run	3 minutes		
Temperature	30 °C		
Flow rate	0.42 mL/min		
Injection volume	1 µL for 100 mm column 1.5 µL for 150 mm column		
Detection	UV 286 nm		

Results and Discussion

Both methods for total phenolic acids and tanshinones regulated in CHP require a traditional column of 4.6 × 250 mm, 5 µm. To get more information from the TCM sample, columns with high peak capacity such as small particle sub-2 µm superficially porous columns are recommended for TCM fingerprint profiling. Peak capacity is a performance measure that describes the number of peaks that can be separated during a gradient run with a certain resolution. Higher peak capacity values are important because of the increasing demand for high-throughput gradients for the separation of complex samples with an unknown number and variety of analytes.

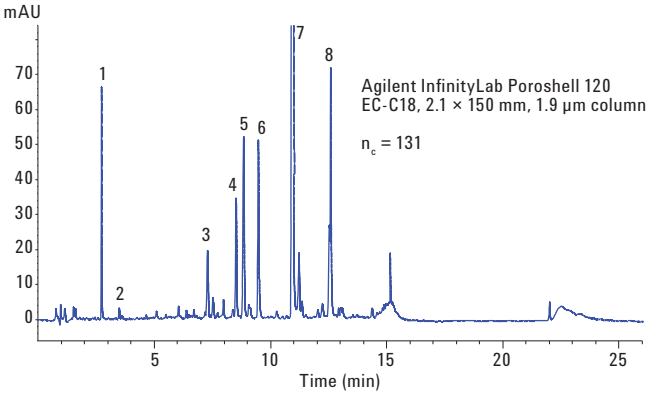

Figure 1. Salvia Total Phenolic Acids fingerprint profiling on an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column. Peaks 5 (rosmarinic acid) and 7 (lithospermic acid B) were identified using reference standards.

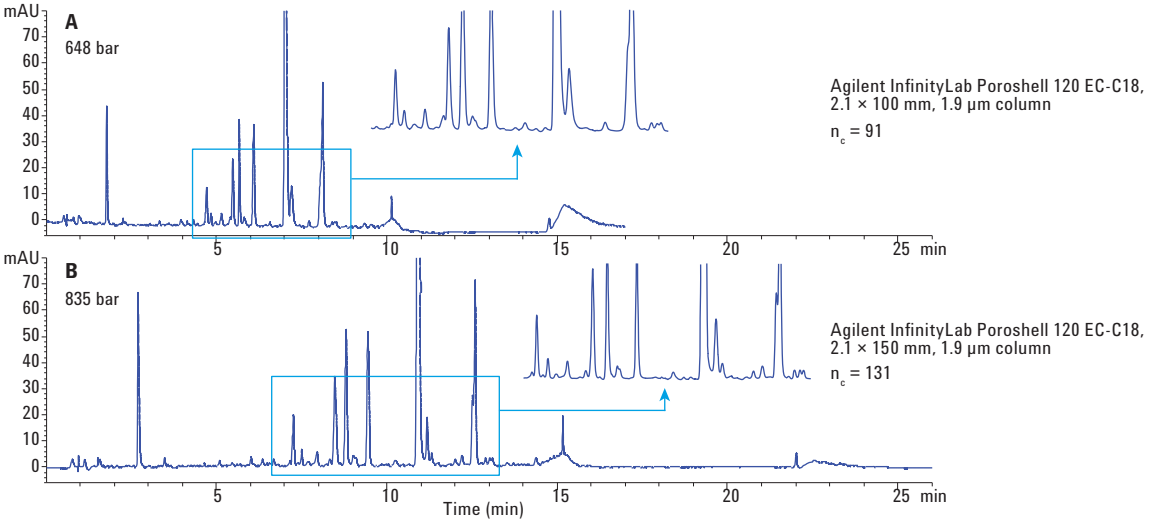

Figure 2. Comparison of the Salvia Total Phenolic Acids fingerprint profiling on Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm and 2.1 × 100 mm columns.

In this application note, the original CHP methods were transferred to Agilent InfinityLab Poroshell 120, 1.9 µm, 150 mm and 100 mm columns. The linear flow rate was doubled to achieve maximum efficiency of sub-2 µm, and the gradient time was adjusted according to the column length and flow rate. Figures 1, 2, 3, and 4 show the chromatograms. The compounds in Figures 2 and 4 marked according to the standard chromatogram in CHP [2] were peaks that should be found in all qualified samples.

The sub-2 µm superficially porous particles columns provided superior performance and fast analysis at UHPLC pressures. Comparing the 2.1 × 100 mm and 2.1 × 150 mm columns, the longer column provided much higher peak capacity and higher resolution, thus providing more information about the samples.

The following equation was used to calculate conditional peak capacity:

$$n_c = (t_{R,n} - t_{R,1})/w$$

where n is the number of peaks used for the calculation, t_R is the gradient time, and w is the average peak width measured at 4σ peak height:

$$w = (W½/2.35) \times 4$$

where W½ = peak width at half height.

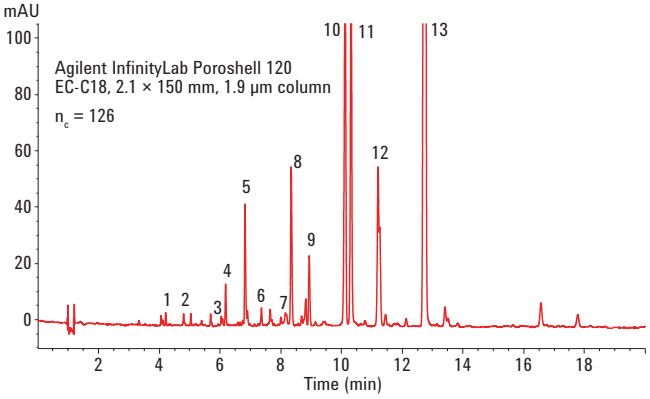

Figure 3. Tanshinones fingerprint profiling on an Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm column. Peaks 10 (cryptotanshinone) and 13 (tanshinone IIA) were identified using reference standards.

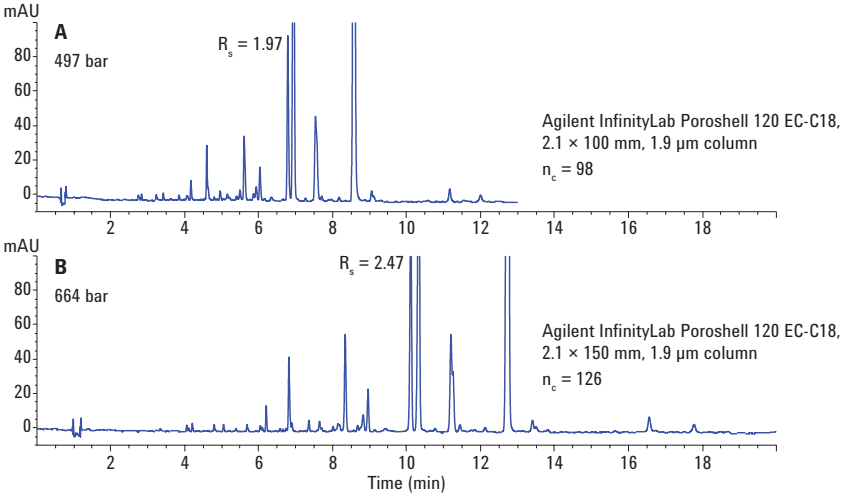

Figure 4. Comparison of the tanshinones fingerprint profiling on Agilent InfinityLab Poroshell 120 EC-C18, 2.1 × 150 mm, 1.9 µm and 2.1 × 100 mm columns.

Conclusions

The sub-2 µm superficially porous particles columns provide superior performance and fast analysis at UHPLC pressures. They are suitable for fingerprint profiling for complex sample analysis of a TCM. A long column gives much greater peak capacity than a short one, providing more information about complex samples.

References

1. Tsai-Hui Lin, Ching-Liang Hsieh, Lin Hsieh. *Chinese Medicine* **5:22** (2010).
2. "Salvia Total Phenolic Acids and Tanshinones" *China Pharmacopoeia* **397**, (2015).

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