Analysis of Echinacoside and Verbascoside in *Cistanche deserticola* Chinese Medicine Using an Agilent Poroshell 120 EC-C18

Application Note

Traditional Chinese Medicine

Author

Rongjie FU

Agilent Technologies (Shanghai) Co. Ltd.

412 Ying Lun Road, Shanghai 200131,

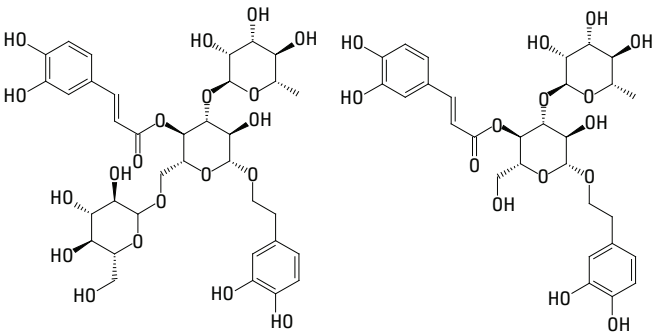
China

Introduction

Due to the complex components in traditional Chinese medicines (TCM), a gradient method with a long time period is often required to separate the target compounds from the complex matrix. HPLC has been an effective method for quality control of TCM applied in the China Pharmacopoeia (CHP).

Cistanche deserticola Y. C. Ma (1960), a commonly used TCM included in the CHP, is prescribed to reinforce the vital function of the kidney and to influence fertility, leukorrhea, and metrorrhagia in women. Phenylethanoid glycosides in *C. deserticola* are the most important active compounds, which have functions of antioxidation, protecting liver and nerves [1]. In the CHP, the amounts of the two main phenylethanoid glycosides of echinacoside and verbascoside (Figure 1) in *C. deserticola* extracts are regulated using HPLC for quality control.

Agilent Technologies



echinacoside verbascoside

Figure 1. Figure 1. Structures of echinacoside and verbascoside

Traditionally, it takes about 35 minutes to analyze the two compounds with the CHP method using a conventional 5 µm particle column. This application note describes a fast quality control method for the analysis of echinacoside and verbascoside using the Agilent 1290 Infinity LC System and an Agilent Poroshell 120 EC-C18, 2.7 µm column. Compared to conventional methods, the rapid method is much faster, and maintains the same performance and quality of separation. In addition, solvent consumption is dramatically reduced.

HPLC conditions

The analysis was performed with the 1290 Infinity LC System including a G4220A Infinity binary pump, G4226A Infinity sampler (ALS), G1316C Infinity Thermostatted Column Compartment (TCC), and G4212A Diode Array Detector SL (DAD).

Conditions

Sample	Extract of Cistanche deserticola
Mobile phase	A, 0.1% (v/v) formic acid; B, methanol
UV	330 nm
TCC temp	30 °C

Conditions for Figure 2

Column	Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 5 µm (p/n 959993-902)	
Gradient	time (min)	%B
	0	26.5
	17	26.5
	20	29.5
	32	29.5
	33	80
Stop time	35 min	
Flow rate	1 mL/min	
Injection volume	10 µL	

Conditions for Figures 3 and 4

Column	Agilent Poroshell 120, EC-C18, 3.0 × 50 mm, 2.7 µm (p/n 699975-902)	
Gradient	time (min)	%B
	0	26.5
	5.67	26.5
	6.67	29.5
	10.67	29.5
	11	80
Stop time	11.67 min	
Flow rate (Figure 3)	0.425 mL/min	
Flow rate (Figure 4)	0.425, 0.85, and 1.7 mL/min	
Injection volume	1.4 µL	

Results and discussion

The original LC method for the analysis of *C. deserticola* used an Agilent ZORBAX Eclipse Plus C18, 4.6 × 150 mm, 5 µm column. Analysis took approximately 35 minutes to separate echinacoside and verbascoside and recondition the column to initial gradient conditions (Figure 2).

By using the 1290 Infinity LC system and a Poroshell 120 EC-C18, 3.0 × 50 mm column, method transfer and optimization were completed quickly and easily. Analysis was accomplished in 12 minutes, while maintaining the same or even better performance for the two target compounds. Since both Agilent columns have similar chemistry, the separation achieved almost the same selectivity (Figure 3).

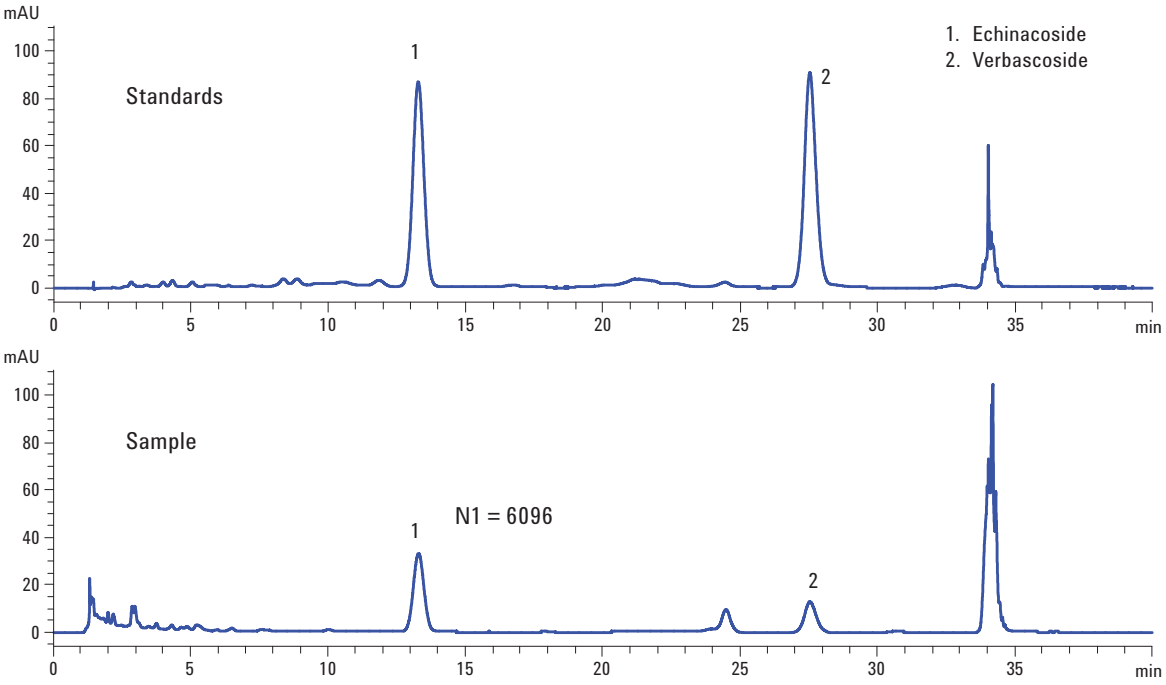


Figure 2. *Figure 2. Echinoside and verbascoside standards, and extract from Cistanche deserticola, analyzed on an Agilent ZORBAX Eclipse Plus C18 4.6 × 150 mm, 5 µm column.*

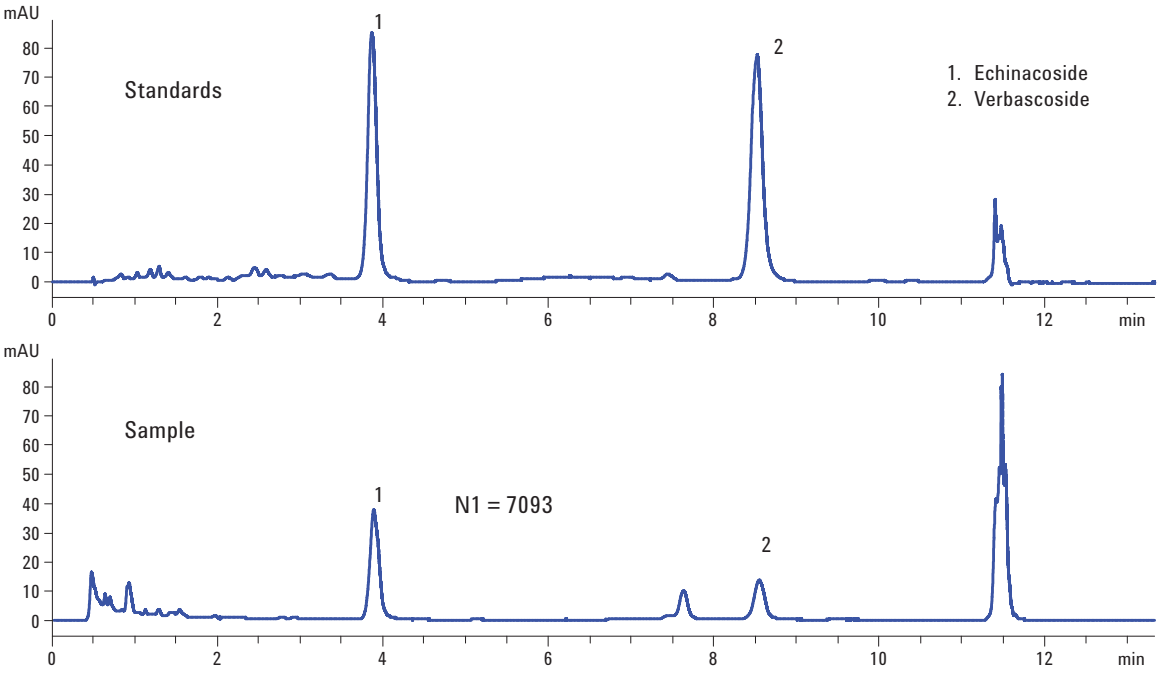


Figure 3. *Echinoside and verbascoside standards, and extract from Cistanche deserticola, analyzed on an Agilent Poroshell 120, EC-C18, 3.0 x 50 mm, 2.7 µm column.*

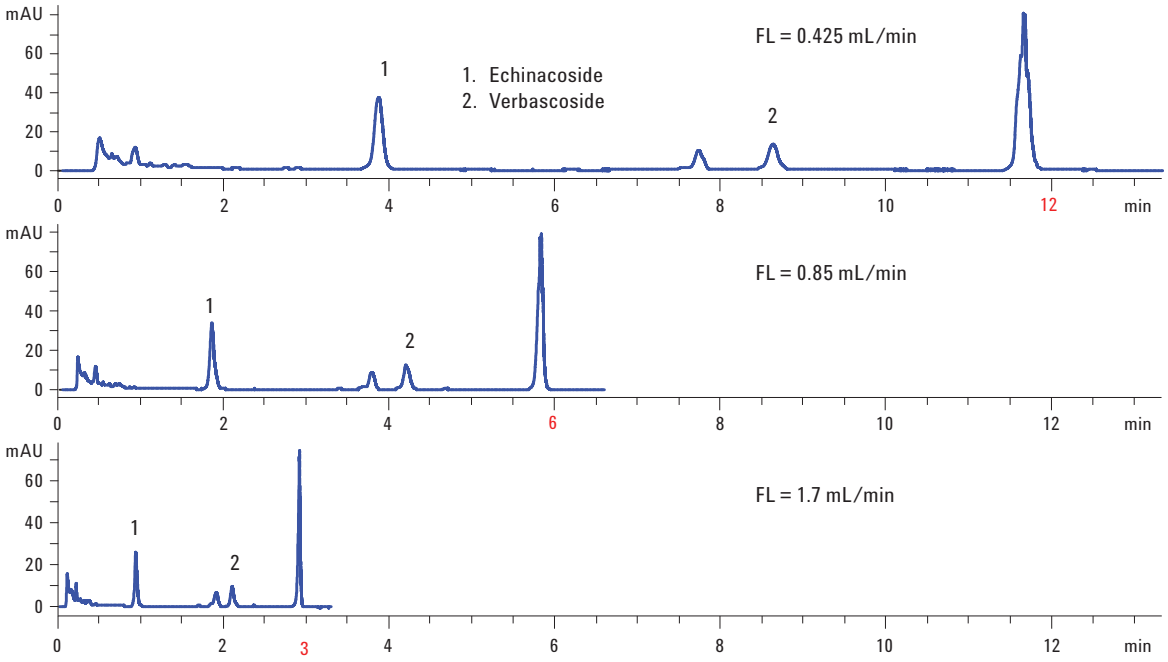


Figure 4. Echinoside and verbascoside standards, and extract from Cistanche deserticola, analyzed on an Agilent Poroshell 120, EC-C18, 3.0 x 50 mm, 2.7 µm column at different flow rates

In addition, solvent consumption was reduced from 35 mL to 5 mL. To take full advantage of the small-particle column, a higher flow rate could be used to further increase the speed of analysis, as shown in Figure 4.

Conclusion

The shorter Poroshell 120 column with 2.7 µm superficially porous particles dramatically reduce the separation time of *C. deserticola* extracts while maintaining a separation similar to that obtained with conventional 5 µm columns. Therefore, quality control of this traditional Chinese medicine is easy and fast when using Agilent Poroshell 120 columns, and time and solvent could be saved for complex analysis of other such medicines.

Reference

1. Chenghua LI, *et al.* RP - HPLC simultaneous determination of four phenylethanoid glycosides in Cistanche tubulosa (Schrenk) Wight.

For More Information

These data represent typical results. For more information on our products and services, visit our Web site at www.agilent.com/chem.

www.agilent.com/chem

Printed in the USA
December 22, 2011
5990-9633EN

Agilent Technologies