

Analysis of notoginseng using C18 chemistries on superficially porous particle columns

Author

Rongjie Fu
Agilent Technologies, Inc.

Abstract

The extracts of total saponins in notoginseng were analyzed by UHPLC using superficially porous particle LC columns with different C18 bonded phases. The regulated method for the analysis of total saponins in extracts of notoginseng in China Pharmacopeia (CHP) was transferred to Agilent InfinityLab Poroshell 120, 3.0 × 100, 2.7 μm columns. The InfinityLab Poroshell HPH-C18 column provided the best resolution among three different C18 phases including SB-C18, EC-C18, and HPH-C18. Compared to the original method run on an InfinityLab Poroshell HPH-C18, 4.6 × 250 mm, 4 μm column, the new method, using an InfinityLab Poroshell HPH-C18, 3.0 × 100 mm, 2.7 μm column, reduced the analysis time by 70 % with almost the same separation.

Introduction

Notoginseng is an herbal medicine used in China extensively since the end of the 19th century. Notoginseng and ginseng have many similar components, as they belong to the same genus, *Panax*. Important components of notoginseng are saponins, flavonoids, polysaccharides, and amino acids. Notoginseng contains high levels of Rb1, Rd, and Rg1 ginsenosides. The China Pharmacopoeia (CHP) requires determination of notoginsenoside R1, ginsenoside Rg1, Re, Rb1, and Rd (Figure 1) using an HPLC method with an analysis time of over 60 minutes¹.

In this Application Note, the original CHP method was first run on an Agilent InfinityLab Poroshell HPH-C18, 4 µm column with superficially porous particles, without any method adjustment. To save time and reduce solvent use, the method was then transferred to shorter columns.

Experimental

Reagents and chemicals

All reagents were HPLC grade or higher. HPLC grade acetonitrile was bought from J. T. Baker (Center Valley, PA, U.S.A.). Water was purified using an ELGA PURELAB Chorus system (High Wycombe, UK). Notoginsenoside R1, Ginsenoside Rg1, Re, Rb1, and Rd were from NIFDC (Beijing, CHINA). Notoginseng total saponins were from a local vendor in China. The test solution was made by dissolving 25 mg of notoginseng total saponins in a 10-mL volumetric flask, which was then diluted with 70 % methanol to volume and shaken.

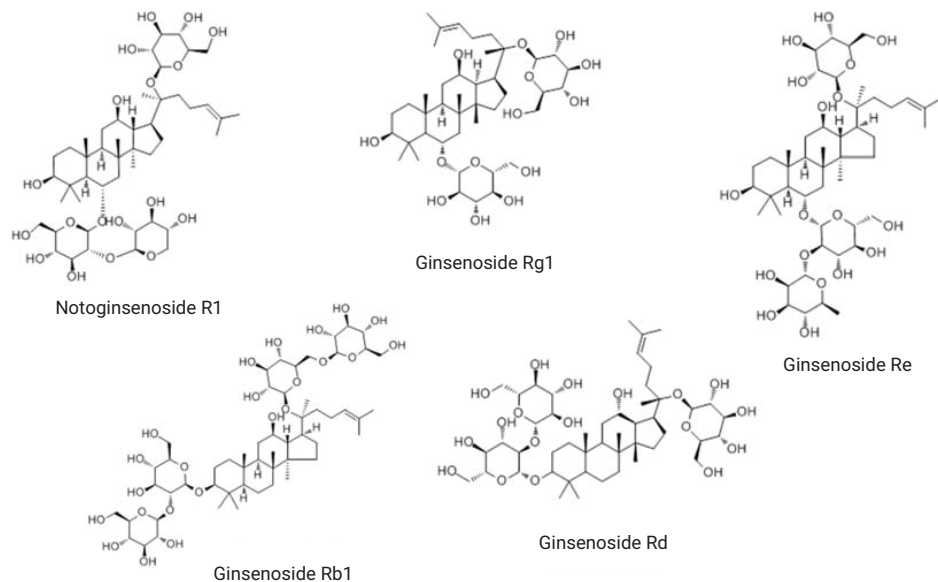


Figure 1. Structures of saponins studied in this Application Note.

Equipment and Materials

- Agilent InfinityLab fittings
 - Column inlet: Agilent InfinityLab Quick Connect fitting (p/n 5067-5965)
 - Column outlet: Agilent InfinityLab Quick Turn fitting (p/n 5067-5966)
- Agilent Captiva Econofilter, PTFE membrane, 13 mm diameter, 0.2 µm pore size (p/n 5190-5265)
- Agilent vial, screw-top, amber, write-on spot, certified, 2 mL, 100/pk (p/n 5182-0716)
- Agilent bonded screw cap, PTFE/red silicone septa (p/n 5190-7024)
- Agilent InfinityLab solvent bottle, amber, 1,000 mL (p/n 9301-6526)
- Agilent InfinityLab Stay Safe cap, GL45, three ports, one vent valve (p/n 5043-1219)
- Eppendorf pipettes and repeater

Instrumentation

- Agilent 1290 Infinity II high speed pump (G7120A)
- Agilent 1290 Infinity II multisampler (G7167B)
- Agilent 1290 Infinity II MCT (G7116B)
- Agilent 1290 Infinity II DAD (G7117B)
- Agilent OpenLab CDS, Rev. C.01.07 SR3 [465]

Results and Discussion

The original CHP method was run on an Agilent InfinityLab Poroshell HPH-C18, 4 μ m, 4.6 \times 250 mm column. This method separated the five target compounds well, but with a long run time of 80 minutes. To save time and solvent, the method was transferred to an InfinityLab Poroshell HPH-C18, 2.7 μ m, 3.0 \times 100 mm column, which is a shorter column with smaller particle size. The results (Figure 2) show comparable separation to the Poroshell HPH-C18, 4.6 \times 250 mm, 4 μ m column. However, analysis time was reduced by 70 %, and solvent use was reduced by over 80 %.

Instrument conditions

HPLC conditions		
Column	Agilent InfinityLab Poroshell HPH, 4.6 \times 250 mm, 4 μ m (p/n 690970-702)	Agilent InfinityLab Poroshell HPH, 3.0 \times 100 mm, 2.7 μ m (p/n 695975-502) Agilent InfinityLab Poroshell 120 EC-C18, 3.0 mm \times 100 mm, 2.7 μ m (p/n 685975-302) Agilent InfinityLab Poroshell 120 SB-C18, 3.0 mm \times 100 mm, 2.7 μ m (p/n 695975-302)
Mobile phase A	Water	
Mobile phase B	Acetonitrile	
Flow rate	1.5 mL/min	0.85 mL/min
Column temperature	25 $^{\circ}$ C	
Injection volume	10 μ L	2 μ L
Detection	203 nm	
Gradient	0–20 minutes: 20 %B 20–45 minutes: 20–46 %B 45–55 minutes: 46–55 %B 55–60 minutes: 55 %B 60–60.1 minutes: 55–95 %B 60.1–70 minutes: 95 %B 70–70.1 minutes: 95–20 %B 70.1–80 minutes: 20 %B	0–6 minutes: 20 %B 6–13.5 minutes: 20–46 %B 13.5–16.5 minutes: 46–55 %B 16.5–18 minutes: 55 %B 18–18.03 minutes: 55–95 %B 18.03–21 minutes: 95 %B 21–21.03 minutes: 95–20 %B 21.03–24 minutes: 20 %B

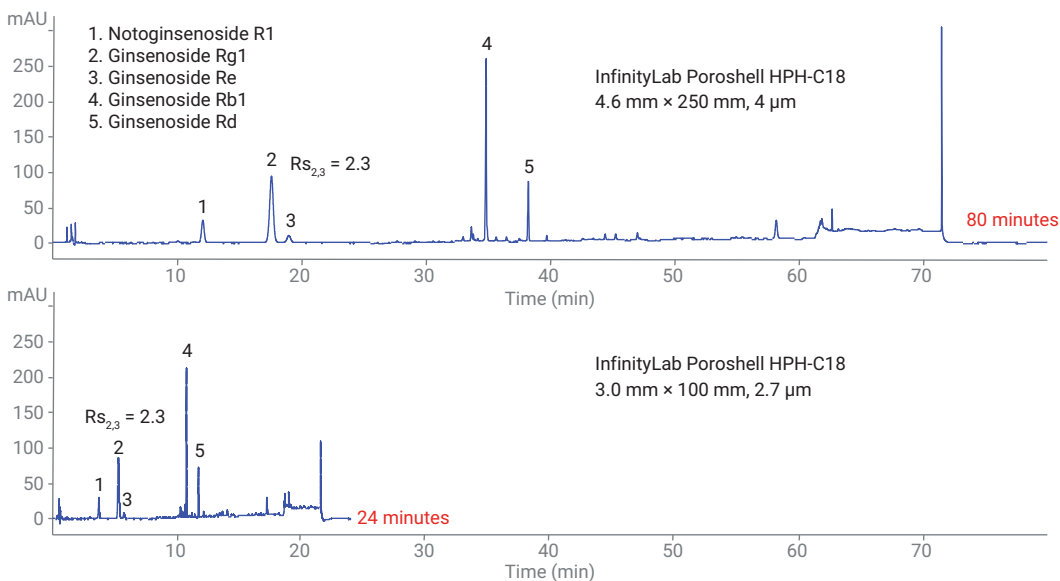


Figure 2. Overlaid chromatograms for total notoginseng saponins analysis on InfinityLab Poroshell HPH-C18, 4.6 \times 250 mm, 4 μ m and 3.0 \times 100 mm, 2.7 μ m columns.

To optimize resolution of all the target compounds, three different C18 columns, including an InfinityLab Poroshell HPH-C18, InfinityLab Poroshell 120 EC-C18, and an InfinityLab Poroshell 120 SB-C18, were compared; Figure 3 shows this comparison. HPH-C18 provides a weak retention for the saponins, but with good resolution. Several minor peaks were well separated with HPH-C18, but in contrast, these coeluted using both the SB-C18 and EC-C18 columns.

Conclusions

The InfinityLab Poroshell HPH-C18, 4 μm column successfully separated saponins in total notoginseng saponins, and the InfinityLab Poroshell HPH-C18, 2.7 μm column significantly saved time and solvents with comparable separation to the original CHP method. An InfinityLab Poroshell HPH-C18 has different selectivity than Poroshell 120 SB-C18 and Poroshell 120 EC-C18, and separated notoginseng saponins better for this application.

Reference

1. Total Notoginseng Saponins, *China Pharmacopoeia* **2015**, 514.

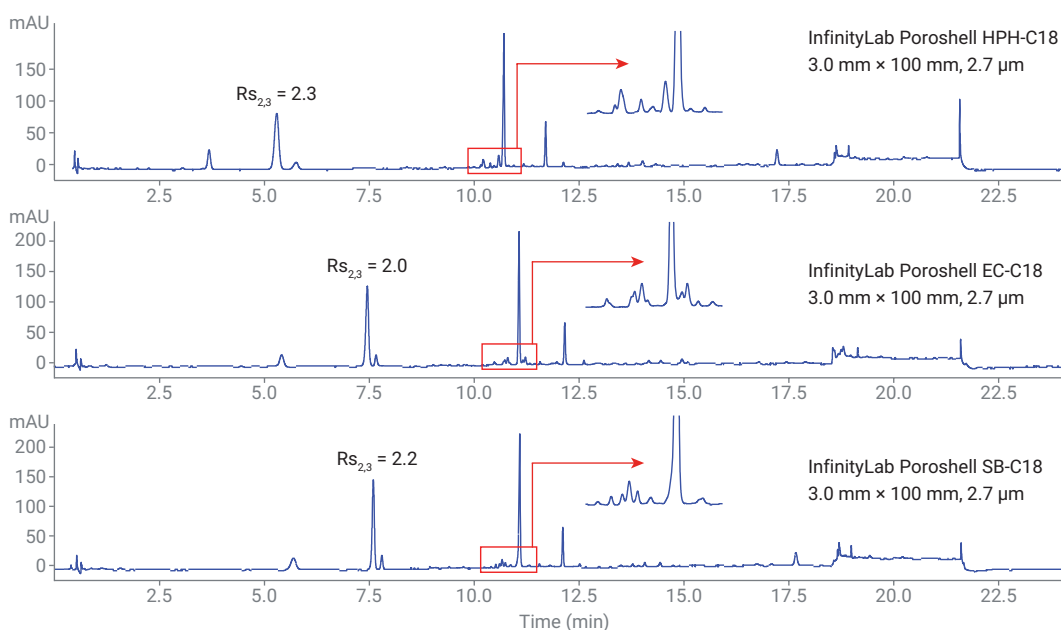


Figure 3. Selectivity comparison of different C18 phases, using InfinityLab Poroshell HPH-C18, 120 EC-C18, and 120 SB-C18 columns.

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Printed in the USA, March 27, 2018
5991-9190EN