Fast method for Ginseng Analyses using Agilent Poroshell 120 Columns Scaled from a Traditional Method

Application Note

Pharmaceutical

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Abstract

The traditional method for analyzing ginseng was scaled from a 4.6 × 150 mm, 5 µm column to an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column. The gradient time required decreased from over 100 min to about 70 min with the same linear velocity, and could be decreased further to below 25 min by increasing the flow rate. In this instance, the pressure exceeded 400 bar at high flow rates, therefore a 600-bar instrument should be used. The time can be further reduced using an Agilent Poroshell 120 EC-C18 or Poroshell 120 SB-C18 3.0 mm × 50 mm column. The fastest method has an 11-min gradient with resolution of 1.57 for the critical pair of compounds, ginsenosides Rg1 and Re. Finally, the new method does not need changes in sample preparation because the column uses 2 µm frits. The pressure is still below 400 bar and can be run on any HPLC instrument.

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Introduction

Asian ginseng is native to China and Korea and is used in various systems of medicine for many centuries. Treatment claims for Asian ginseng are numerous and the herb is used to support overall health and boost the immune system. Traditional and modern uses of ginseng include: improving the health of people recovering from illness; increasing a sense of well-being and stamina; improving both mental and physical performance; treating erectile dysfunction, hepatitis C, and symptoms related to menopause; lowering blood glucose; and controlling blood pressure. The root of Asian ginseng contains active chemical components called ginsenosides that are thought to be responsible for the herb's medicinal properties [1].

In the Chinese Pharmacopeia, an HPLC method is listed to measure amounts of the three main ginsenosides Rg1, Re and Rb1 in ginseng [2]. The original method uses a traditional 4.6 mm × 150 mm, 5 µm column and applies a long gradient of 100 minutes. A rapid analysis method is necessary to increase the work throughput and reduce lab costs.

In this study, the original method was scaled to both Agilent Poroshell 120 EC-C18 and Poroshell 120 SB-C18 columns. The Agilent Poroshell 120 2.7 µm columns are packed with superficially porous materials, which make the separation fast and achieves performance similar to sub-2-µm totally porous materials. The method was scaled for column length, column id, and sample size. The linear velocity was maintained on the smaller column.

Experimental

The 1200 Series SL LC system includes a binary pump, a thermostatted column compartment (TCC), a high performance autosampler and a diode array detector (DAD).

The columns used in the application are:

- Agilent ZORBAX StableBond C18, 4.6 mm × 150 mm, 5 µm (p/n: 883975-902)
- Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm (p/n: 959993-902)
- Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm (p/n: 695975-302)
- Agilent Poroshell 120 EC-C18, 3.0 mm × 50 mm, 2.7 µm (p/n: 699975-302)
- Agilent Poroshell 120 SB-C18, 3.0 mm × 50 mm, 2.7 µm (p/n: 689975-302)

The ginseng sample was made using following steps:

1. Weigh 1.0 g of the dried powder.
2. Degrease with Soxhlet extractor using 50 mL ethyl ether for 3 h.
3. Filter after cooling down, discard the liquid phase, then dry the residues.
4. Put the residues in a 25-mL flask with filter paper. Add 20 mL of water-saturated n-butanol and extract for 30 min with a supersonic extractor.
5. Filter the extraction and collect 10 mL of the liquid of n-butanol.
6. Evaporate the n-butanol using a water bath and add 2 mL of methanol to the residue to dissolve.
7. Filter the final sample with a 0.45-µm regenerated cellulose membrane filter (p/n: 5064-8221) before injecting into HPLC for analysis.

Results and Discussion

The original ginsenosides separation method separating Rg1, Re and Rb1 ginseng was repeated on an Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm and an Agilent ZORBAX SB-C18, 4.6 mm × 150 mm, 5 µm column. Theoretical plates of ginsenosides Rg1 on both columns were more than 9000 and the resolution was around 2.0, which is sufficient for the determination of three ginsenosides. The time for a single run was more than 100 min as shown in Figure 1.

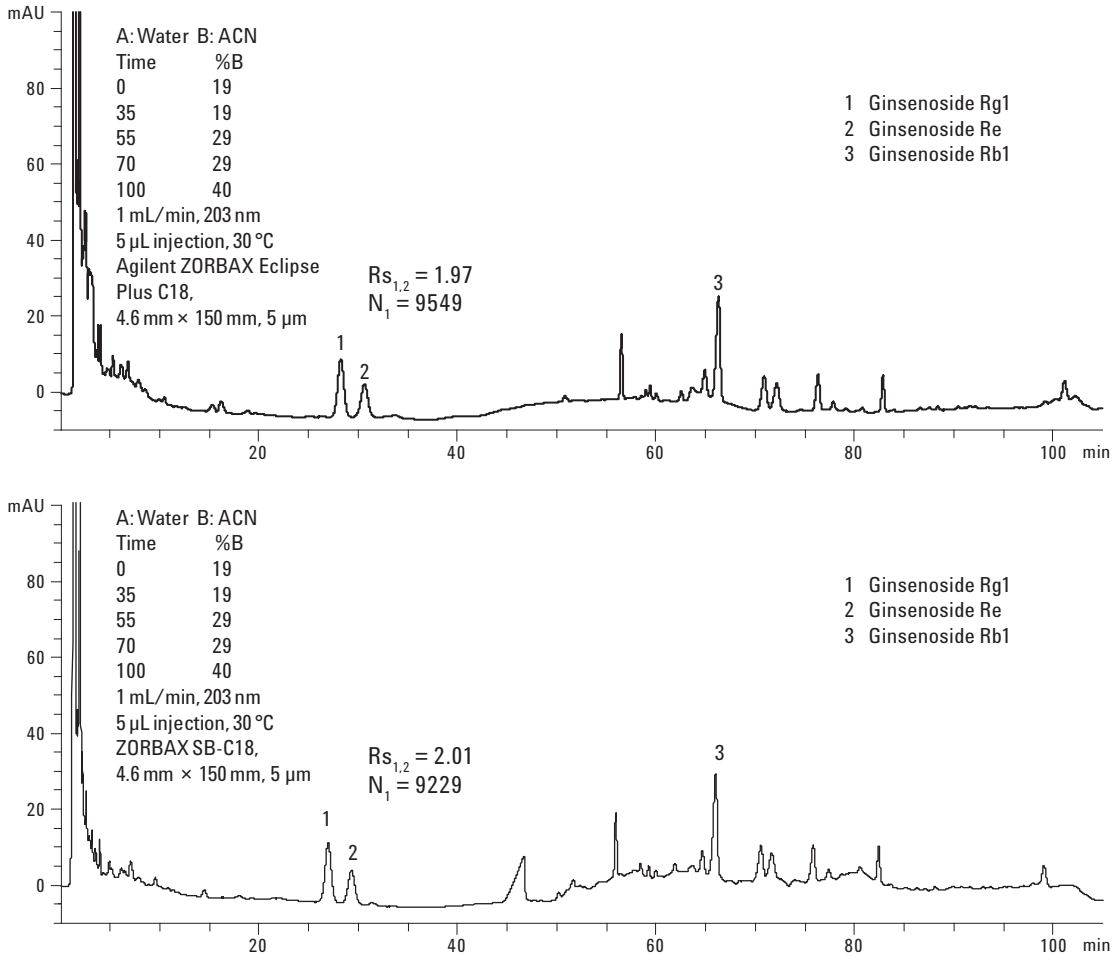

Figure 1. Original method for analyzing ginseng on an Agilent ZORBAX Eclipse Plus C18, 4.6 mm × 150 mm, 5 µm column and an Agilent ZORBAX SB-C18, 4.6 mm × 150 mm, 5 µm column.

The original method was then transferred to an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column. The flow rate was changed according to the below equation to maintain the same linear velocity.

Equation 1: $F_1/(r_1)^2 = F_2/(r_2)^2$

where

F_1 is the flow rate of original column
F_2 is the flow rate of new column
r_1 is the radius of original column
r_2 is the radius of new column

The gradient time is proportional to the column length while maintaining the original separation. Since the original column is 4.6 mm × 150 mm, using a 3.0 mm × 100 mm column at the same linear velocity shortens the gradient time by 100/150. The injection volume is decreased properly to avoid sample overload. The analysis time, therefore, decreased from 100 min to 66.7 min as shown in Figure 2. The backpressure of 174 bar is acceptable for a 400-bar HPLC. The chromatogram shows better resolution and theoretical plates than the original method.

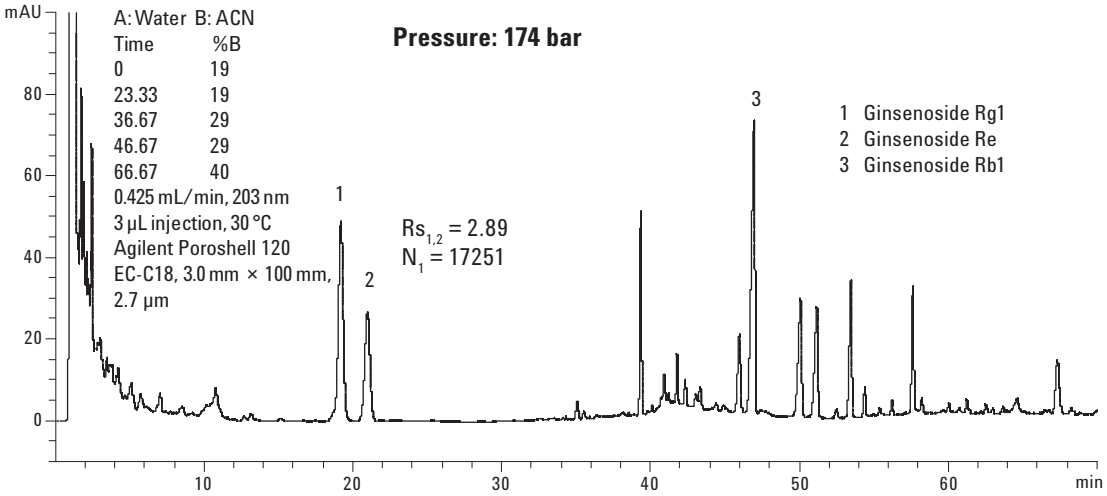

Figure 2. Chromatogram using an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column at normal flow rate.

The new method saves one-third of the original analysis time, and is a substantial improvement. The analysis time can be further shortened by increasing the flow rate and decreasing the gradient time. The gradient time is proportional to the flow rate using the same column. Therefore the analysis time reduces to half that with the new method when using twice the original flow rate (increasing from 0.425 mL/min to 0.85 mL/min). The cycle time is further reduced by using three times the original flow rate (Figure 3) with some loss in resolution and theoretical plates, which are still better than those on traditional columns. This is possible because the Van Deemter curve of the superficially porous Poroshell 120 2.7 µm particles is similar to columns with 1.8 µm particles. The efficiency performance of the Poroshell column does not decrease at high flow rate, compared to that of columns with 1.8 µm packing. At three times the original flow rate the pressure is about 3 times higher than the original 174 bar and is about 485 bar. This pressure is fine for the column, which can be used up to 600 bar, but does require an HPLC or UHPLC with a pressure limit of at least 600 bar, such as the Agilent 1260 Infinity LC.

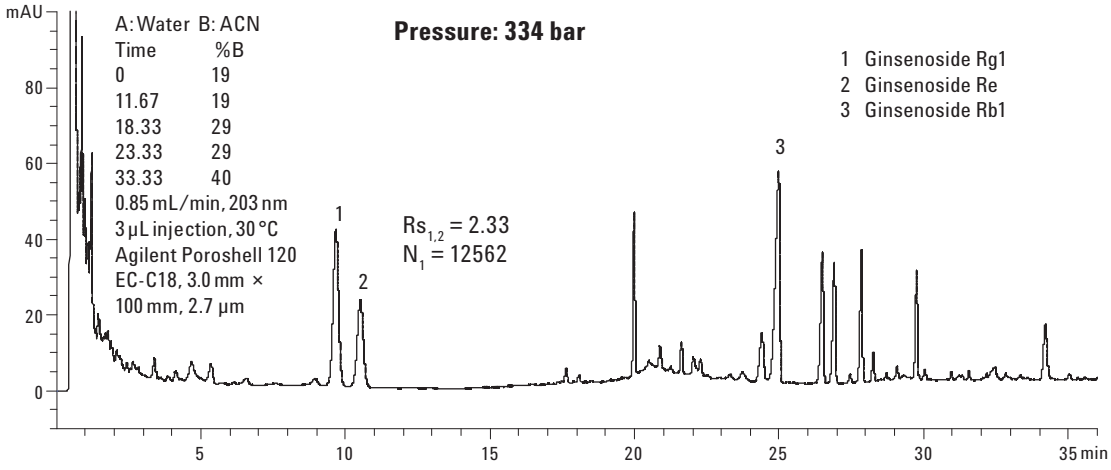

Figure 3. Chromatograms at higher flow rate using an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column. (Continued)

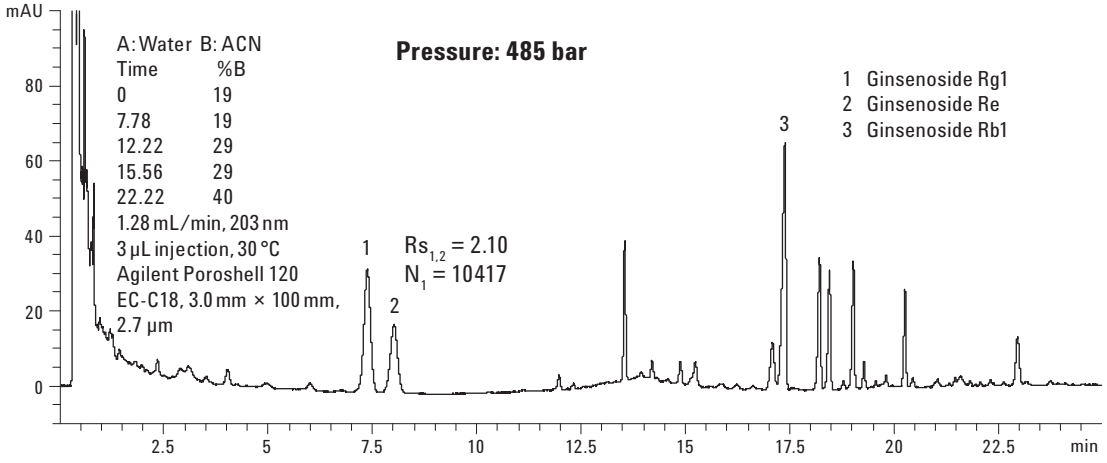

Figure 3. Chromatograms at higher flow rate using an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column.

Significant time savings is found in the overlaid chromatogram (Figure 4) using an Agilent Poroshell 120 EC-C18, 3.0 x 100 mm, 2.7 µm column with different flow rates.

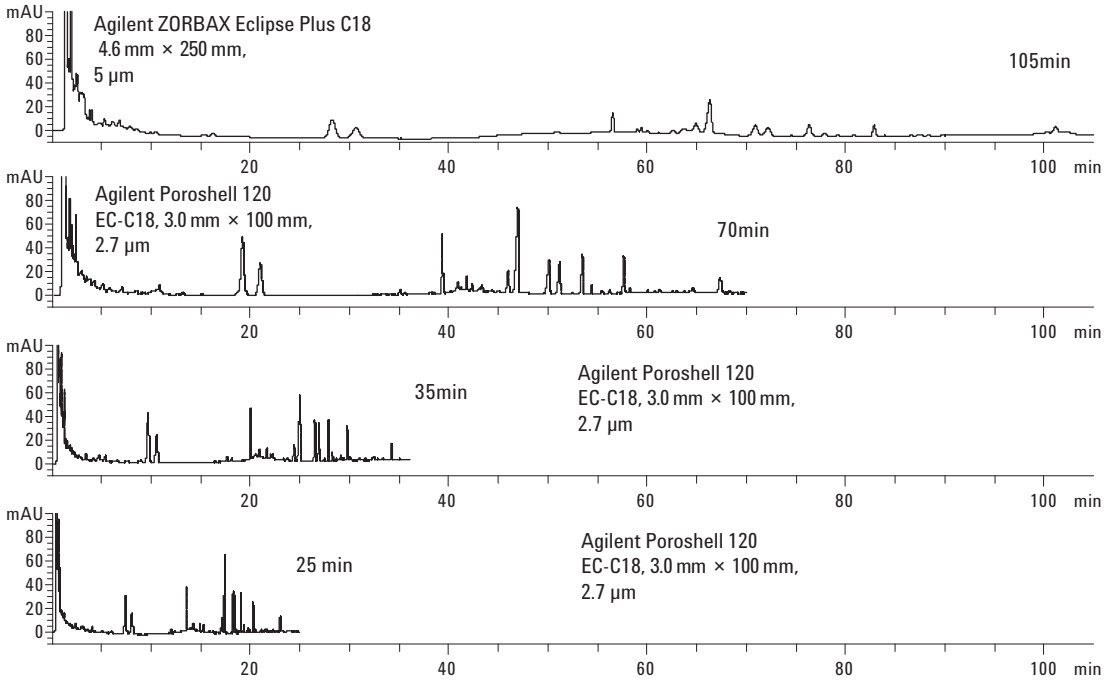

Figure 4. Overlaid chromatograms using an Agilent ZORBAX Eclipse Plus C18, 4.6 mm x 150 mm, 5 µm column and an Agilent Poroshell 120 EC-C18, 3.0 mm × 100 mm, 2.7 µm column at different flow rates.

The method developed on an Agilent Poroshell 120, 3.0 mm × 100 mm column saved three-fourths (75%) of the original analysis time, however the separation time can be further shortened using a shorter column. The method was then transferred to an Agilent Poroshell 120, 3.0 mm × 50 mm column. The separation requires 23 min with the Rs of ginsenosides Rg1, Re close to 2.0. The separation could be complete in only 11 min (about one-tenth of original time) when the flow rate was doubled with some compromise of resolution and performance, which is still acceptable for quantitative measurement.

It is important to note that the pressure is only around 200 bar, which is far below the limit of a 400-bar instrument. Theoretically, this fast separation could be run on a 400-bar instrument, but for the delay volume and post column volume, the instrument should be optimized and HPLC condition adjusted to achieve the ideal separation.

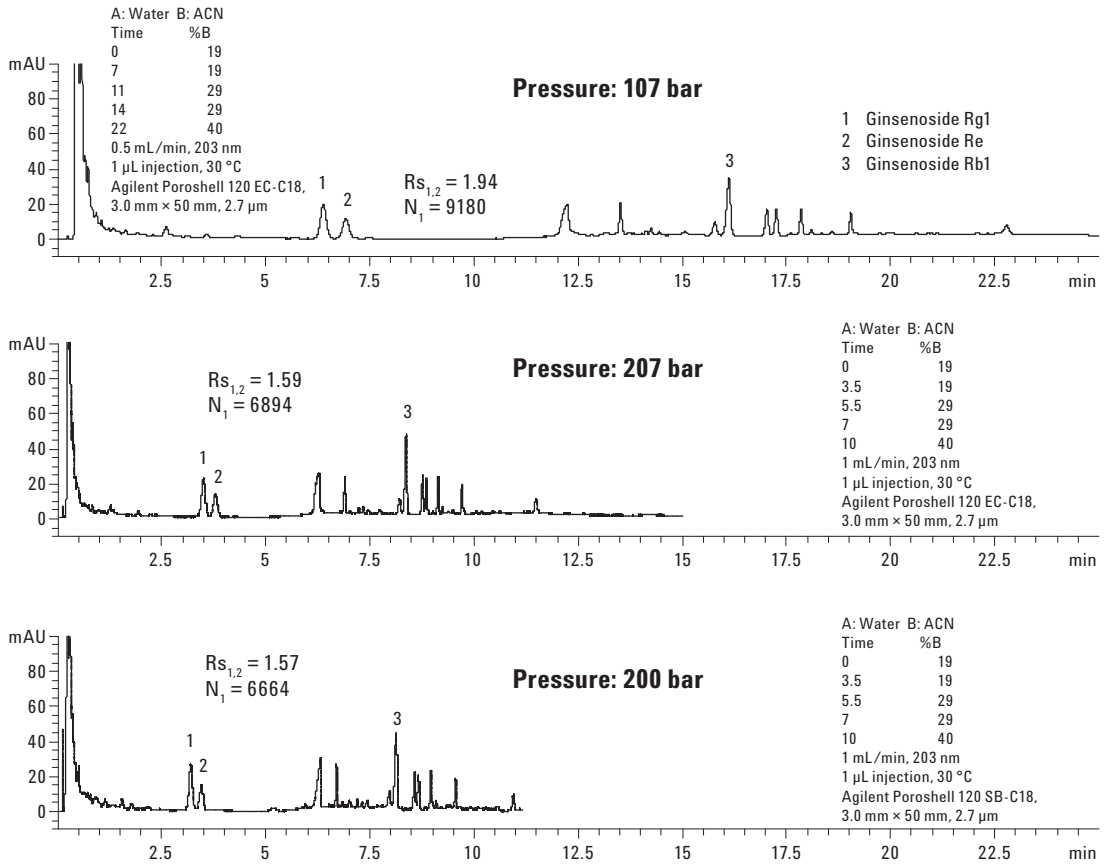

Figure 5. Chromatograms of ginseng using an Agilent Poroshell 120 EC-C18, 3.0 mm × 50 mm, 2.7 µm and an Agilent Poroshell 120 SB-C18, 3.0 mm × 50 mm, 2.7 µm columns

Conclusion

The method for the analysis of ginsenosides in ginseng was successfully transferred from a traditional 4.6 × 150 mm, 5 µm column to a Poroshell 120 column with substantial time savings and no compromise in resolution. The best method choice depends on what time is desired and what HPLC's are available for use. The superficially porous 2.7 µm particle columns provide similar performance to that of the totally porous sub-2-µm columns but with lower pressure. Due to the low pressure, a 400-bar instrument can run this method. A higher flow rate allows faster separations on a UHPLC, up to the 600-bar pressure limit of the column.

Reference

1. http://nccam.nih.gov/health/asianginseng/
2. Ginseng, China Pharmacopoeia, edition 2010

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