Faster LC Analysis of Notoginseng Total Saponins Using an Agilent Poroshell 120 EC-18

Application Note

Traditional Chinese Medicine

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Abstract

The traditional method for analyzing notoginseng total saponins was transferred from an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column to an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm column. Gradient time decreased from 60 minutes to 18 minutes. The transferred method was fast, with higher efficiency and a 2.11 resolution for the critical pair of compounds, ginsenosides Rg1 and Re. In addition, backpressure was below 270 bar and so the analysis could be run on a traditional HPLC instrument.

Introduction

Panax notoginseng, also known as San Qi, is a plant of the Araliaceae family. It is a traditional Chinese herb well-known for its therapeutic abilities to stop hemorrhage [1], to influence blood circulation, and to act as a tonic. *P. notoginseng* contains about 8–12% by weight of saponins. Total saponins of *P. notoginseng*, the major bioactive components, are used to treat coronary heart disease, cardiac angina, apoplexy, and atherosclerosis [2, 3]. However, notoginseng total saponins contain several kinds of active components such as notoginsenoside R1, ginsenoside Rg1, Rb1, Re, and Rd. The analysis of saponins is important for evaluating the quality of notoginseng and its Chinese medicine preparations.

Traditionally, the HPLC run time is greater than 60 minutes for the analysis of notoginseng total saponins with the China Pharmacopeia method using a conventional LC column [4]. Agilent Poroshell 120 EC-C18, 2.7 µm columns are packed with superficially porous materials, which deliver fast separation and achieve performance similar to sub-2 µm totally porous materials, but with lower pressure. This application note describes a fast quality control method for the analysis of notoginsenoside R1 and Ginsenosides Rg1, Re, Rb1, and Rd using the Agilent 1290 Infinity LC System and a Poroshell 120 EC-C18 column. Compared to conventional methods, the rapid method is much faster, with better performance, and quality of separation. In addition, solvent consumption is dramatically reduced.

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Experimental

Analyses were performed on an Agilent 1290 Infinity LC System consisting of a binary pump (G4220A), a thermostatted column compartment (TCC, G1316C), an autosampler (G4226A), and a diode array detector (DAD, G4212A).

Columns

Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm (p/n 959990-902)
Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm (p/n 697975-902)

Compounds

Compounds of interest are shown in Figure 1, with their respective structures. They were dissolved in 70% methanol aqueous solution at 2.5 mg/mL. Notoginseng total saponins were purchased from a local TCM store. Twenty five mg of the sample powder were transferred to a 10 mL volumetric flask and a 70% methanol aqueous solution was added to dissolve and dilute to volume. This solution was then filtered through a 0.45 µm regenerated cellulose membrane filter (p/n 5064-8221) and injected directly into the HPLC system.

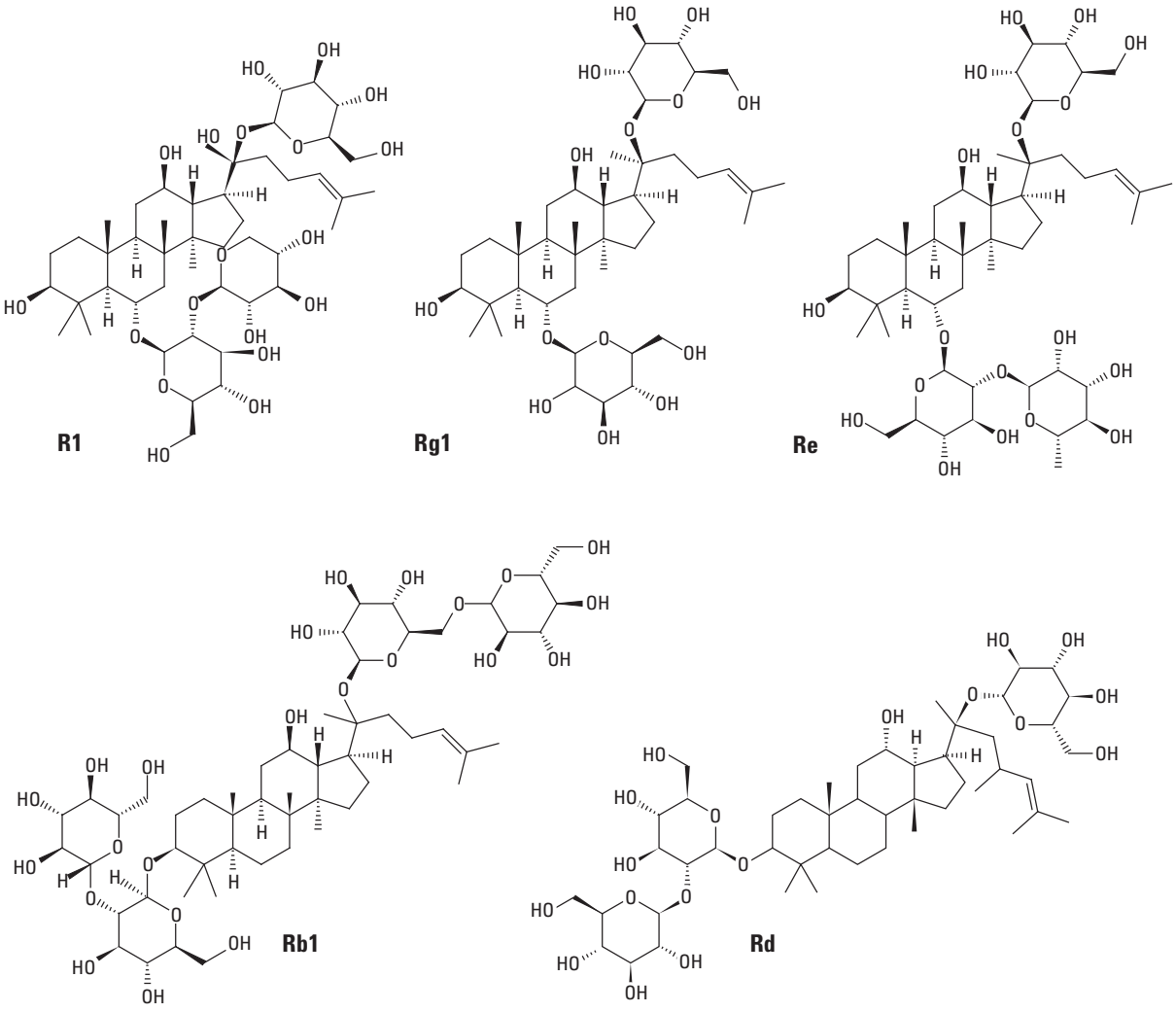

Figure 1. Structure of notoginsenoside R1 and ginsenosides Rg1, Re, Rb1 and Rd.

Results and Discussion

The original separation method of notoginseng total saponins published in the 2010 Chinese Pharmacopoeia was repeated on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column. It took approximately 60 minutes to separate notoginsenoside R1 and ginsenosides Rg1, Re, Rb1, and Rd. Compounds of interest were baseline separated with excellent peak shape. The Agilent Poroshell 2.7 µm particle columns provided similar performance to that of totally porous sub-2 µm columns, but with lower pressure. By using an Agilent Poroshell 120 EC-C18 4.6 × 75 mm, 2.7 µm column, method transfer and optimization were completed quickly. As shown in Figure 2, the analysis time decreased from 60 minutes to 18 minutes, while achieving better resolution for the critical pair Rg1 and Re, and better theoretical plates for Rg1, which exceeded the requirement of the 2010 Chinese Pharmacopoeia (N>6000). The pressure was less than 270 bar, which is quite acceptable for a 400-bar HPLC when using an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm column. In addition, solvent consumption can be significantly decreased, thereby lowering costs.

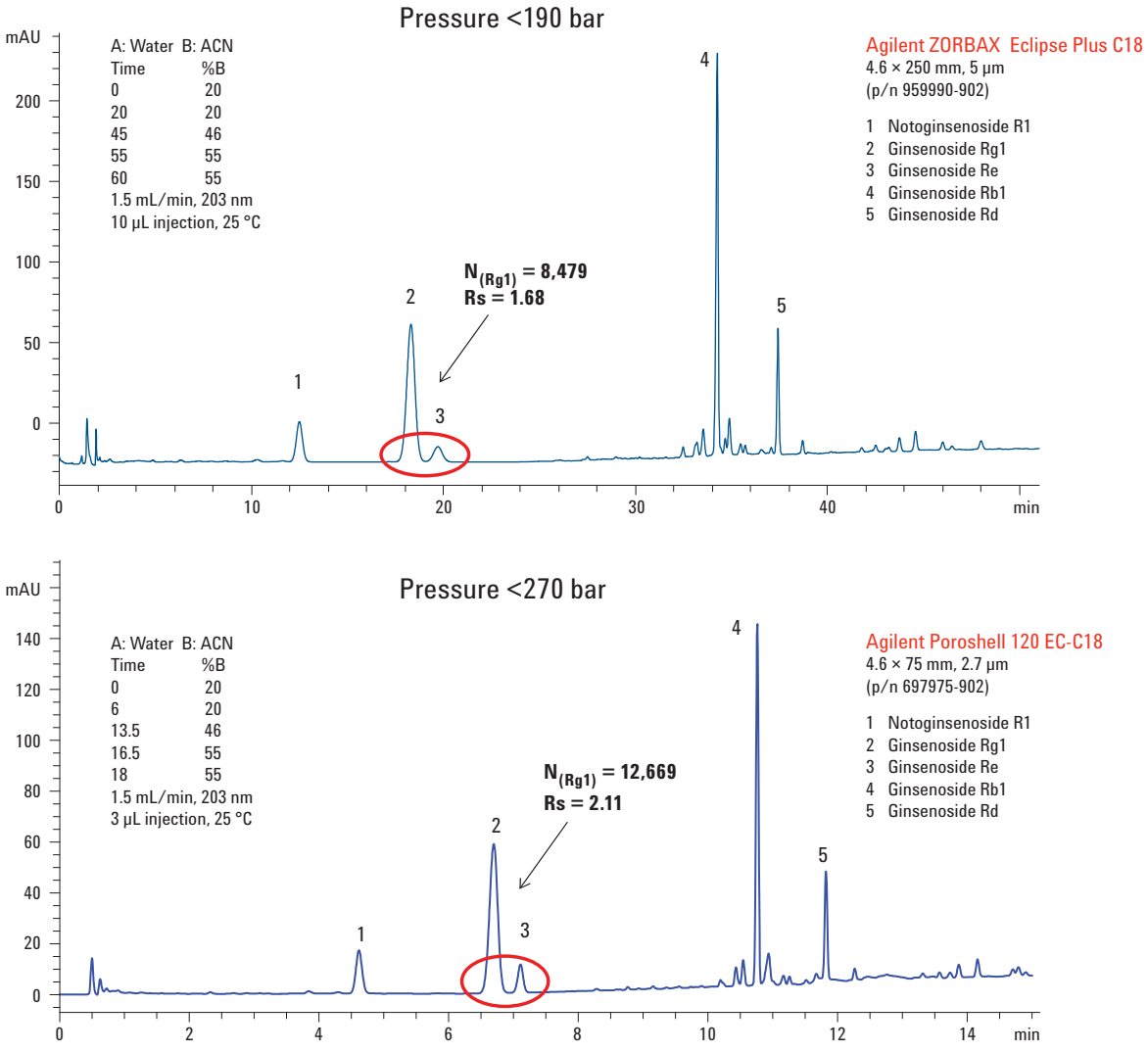

Figure 2. Overlaid chromatograms of notoginseng total saponins using an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm and an Agilent Poroshell 120 EC-C18, 4.6 × 75 mm, 2.7 µm.

Conclusions

The traditional method for analyzing notoginseng total saponins was reproduced successfully on an Agilent ZORBAX Eclipse Plus C18, 4.6 × 250 mm, 5 µm column. The shorter Agilent Poroshell 120 EC-C18 column can greatly reduce the analysis time and provide better separation and peak shape, and thereby substantial time and cost savings. The Poroshell 120 column can exceed the requirements of the 2010 Chinese Pharmacopoeia for notoginseng total saponins analysis. It is well suited for evaluating the quality of notoginseng and its Chinese medicine preparations.

References

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