Speed up the LC Analysis of Notoginsenoside R1 and Ginsenosides Rg1, Re, and Rb1 in Compound TCM Using the Agilent 1290 Infinity LC system and ZORBAX RRHD 1.8 µm Column

Application Note

Pharmaceuticals

Introduction

Ginsenosides belong to a class of steroid glycosides found exclusively in the plant genus Panax (ginseng). Ginsenosides have been the target of research, because they are viewed as the active compounds behind the claims of ginseng's efficacy. As quality indicators, determination of those compounds is required for quality control methods of most ginsenosides containing TCM and neutraceutical products. Traditionally, it takes almost two hours to analyze these compounds with the China Pharmacopeia method using conventional HPLC. This Application Note describes a fast quality control method for the analysis of notoginsenoside R1 and Ginsenosides Rg1, Re and Rb1 using the Agilent 1290 Infinity LC system and an Agilent ZORBAX RRHD 1.8 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 also dramatically reduced.

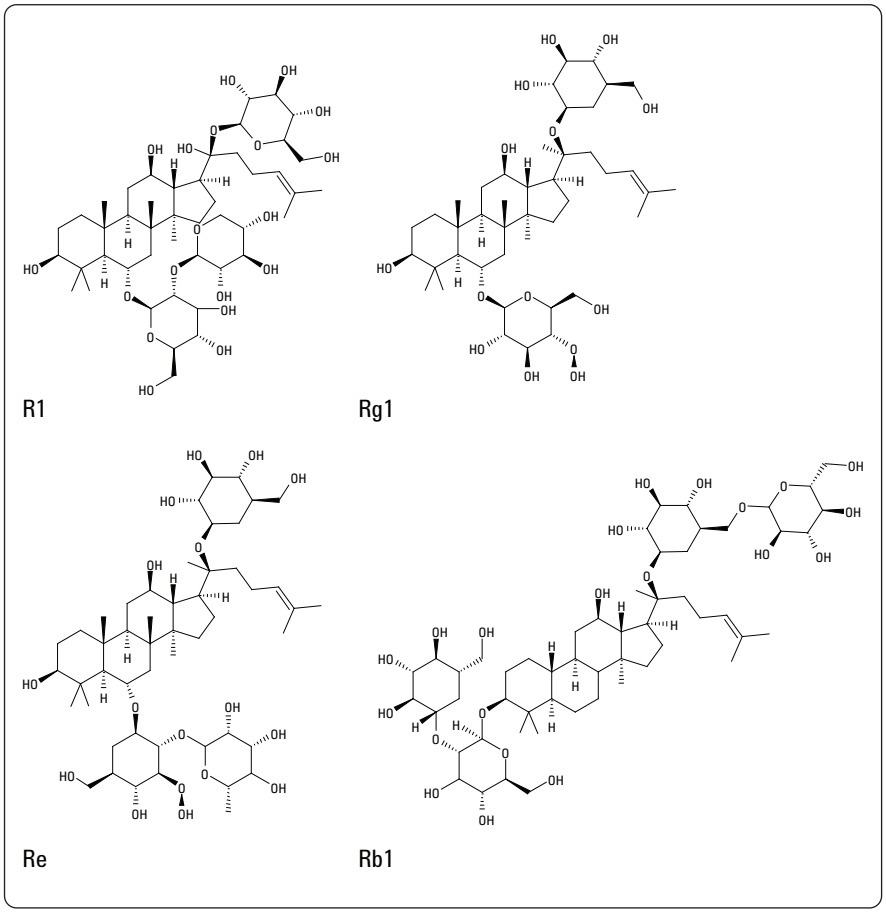

Figure 1
Structure of notoginsenoside R1 and Ginsenosides Rg1, Re and Rb1.

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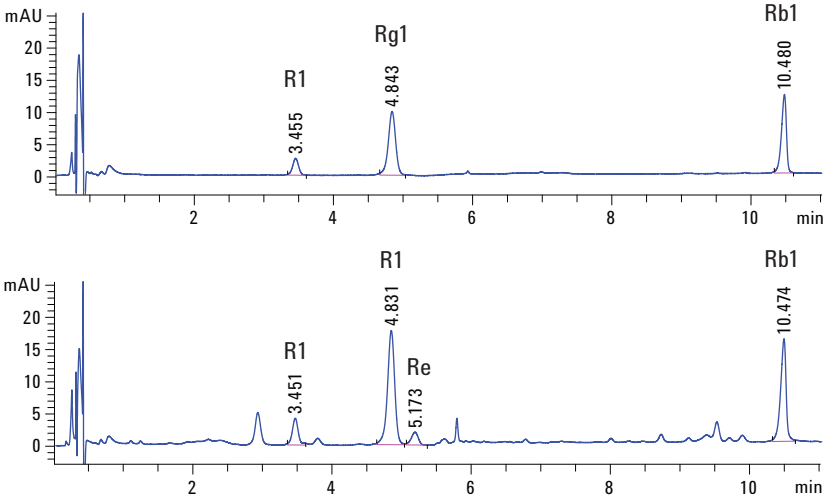
Results and Discussion

Compound TCM products normally consist of multiple active components that come from different plants. Because the matrix of those kinds of products is very complex, separation often requires a gradient method with a long period of time. In this Application Note, an extract of Fufang Dansen dripping pill, which is a compound TCM product, was analyzed. The original LC method used a 4.6 mm × 150 mm, 5 µm column, and took almost 120 mins to separate R1, Rg1, Re and Rb1, and recondition the column to initial gradient conditions. By using the Agilent 1290 Infinity LC system and a 2.1 mm × 50 mm RRHD column, method transfer and optimization were completed quickly. The analysis was completed in 12.6 min, while maintaining the same or even better resolution for the critical peak pair Rg1 and Re. Since the column chemistry did not change from 5 µm to 1.8 µm (both used Stablebond C18 chemistry), the separation achieved the same selectivity. In addition, solvent consumption was reduced from 120 mL to 5 mL.

Configuration

The Agilent 1290 Infinity LC system consisted of:

- Agilent 1290 Infinity Binary Pump with Integrated Vacuum Degasser (G4220A)
- Agilent 1290 Infinity Autosampler (G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (G1316C)
- Agilent 1200 Diode Array Detector (G1315C)



Upper:	Chromatogram for standards; Bottom: chromatogram for sample
Condition:	Sample: extract of Fufang Danshen dripping pills, 0.4 mL/min,
Gradient:	0 min-19% ACN, 81% H_2O, 4.5 min-20% ACN, 5 min-25%ACN, 10 min-30% ACN, 10.50 min-30% ACN, 10.60 min-90% ACN,
Stoptime:	11.60 min.
Posttime:	1.00 min. 203 nm,
Column temperature:	20.00 °C
Column:	Agilent ZORBAX Stablebond C18 RRHD 2.1 mm × 50 mm, 1.8 µm

Figure 2
Separation of R1, Rg1, Re and Rb1 achieved with an Agilent 1290 Infinity LC system and Agilent ZORBAX StableBond C18, 1.8 µm RRHD column.

Conclusion

The shorter STM column reduces the separation time. The low delay volume of the Agilent 1290 Infinity LC system allows faster re-equilibration after gradient, especially when using a narrow bore column at a lower flow rate. The combination of those two factors allows fast analysis of the complex components of the TCM product, with much lower solvent consumption.

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