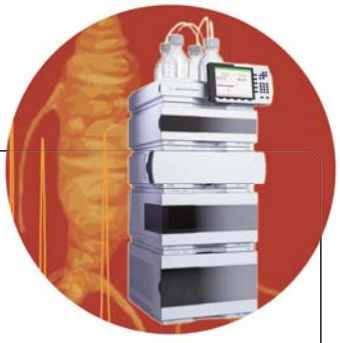
Analysis of TCM injections using the Agilent 1290 Infinity LC system

Application Note

Traditional Chinese Medicine



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Abstract

An ultra high performance liquid chromatography (UHPLC) method using an Agilent 1290 Infinity LC system with an Agilent 1290 Infinity Diode Array Detector (DAD) was developed for the separation of Shenmai injections. Good separation of complex systems was achieved by connecting two columns: an Agilent ZORBAX Eclipse Plus C18 column (2.1 mm × 100 mm, 1.8 µm) and an Agilent ZORBAX SB C18 (2.1mm × 50 mm, 1.8 µm) column. These were chosen for their separation efficiency and good chromatogram peak shape. The results demonstrated that this analytical method was simple, sensitive and reliable for rapidly analyzing TCM injections, and is helpful in comprehensively evaluating the quality of the TCM injection.

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Introduction

Traditional Chinese Medicine (TCM) injections, also known as Chinese herbal injections, have been used for several decades in China. However, evidence of adverse drug reactions (ADR) through the years, have attracted attention to TCM injections.

The ADRs have several causes.[1] The consequences of these ADRs have led to discussions regarding the safety of TCM, because many complex components of TCM injections not yet been identified.

However, TCM injections have the advantage of higher bioavailability, faster therapeutic effects, and low cost. Eliminating TCM injections is not the best solution for reducing health issues caused by ADR. Therefore, government agencies and manufacturers have held several conferences and summits discussing how to improve safety standards, through better component understanding and stricter quality control.

A Shenmai injection composed of *Radix Ginseng Rubra* (red ginseng) and *Radix Ophiopogonis* (maidong) is widely used in China to cure the syncope caused by Yin and Qi deficiences and coronary heart disease. Some ADR reports related to Shenmai injection show an urgent need to improve the quality control level of these injections. More research regarding component elucidation is necessary. UHPLC reduces the issues involved with the separation of such complex matrices.

Research on *Radix Ophiopogonis* has shown that the main active components are isoflavoids (for example, Ophiopogonone A-F)[2] and saponins (for example, Ophiopogonin D). The methods discussed in several scientific papers published for the analysis of ginseng[3], ophiopogonis and Shenmai injections require long analysis times[2,4].

In this Application Note, a fast analysis method with good separation of the complex components of Shemmai injections was achieved with an Agilent 1290 Infinity UHPLC system. Since research on the therapeutic effects of these injections should be based on good separations, the method published here is applicable. It is a method for improved quality control and for high throughput research.

Experimental

Equipment:

For development of the UHPLC method, an Agilent 1290 Infinity UHPLC system with the following modules was used:

- Agilent 1290 Infinity Binary Pump with integrated vacuum degasser (p/n G4220A)
- Agilent 1290 Infinity High Performance Autosampler (p/n G4226A)
- Agilent 1290 Infinity Thermostatted Column Compartment (p/n G1316C)
- Agilent 1290 Infinity Diode Array Detector SL with Max-Light flow cell (1.0 µL volume, 10 mm path length) (p/n G4212A)
- Agilent ChemStation B.04.02 for data acquisition and evaluation
- Agilent ZORBAX RRHD Eclipse Plus C18 column 2.1 mm × 100 mm, 1.8 µm (p/n 857700-902)
- Agilent ZORBAX RRHD SB C18 column 2.1 mm × 50 mm, 1.8 µm (p/n 959758-902)
- Two columns were connected via a capillary 50 mm × 0.12 mm (p/n G1316-87312)

Chemicals and materials

HPLC-grade acetonitrile, acetic acid and ammoniom acetate were purchased from J&K Chemical LTD. Pure water was obtained from a Millipore pure water system. The reference standards of 20(R)-Propanaxadiol, 20 (R)-Propanaxatriol, Ginsenoside Re, Rg1, Rb1, Rc were purchased from the National Institute for the Control of Pharmaceutical and Biological Products (NICPBP). Ginsenoside Rb2, Rb3, Rg3, Rh2, Rf, F11 were kindly provided by the customer. All the standards were dissolved in methanol to make 1 mg/mL solutions.

Shenmai injection and ophiopogonis extraction was provided by customer.

Sample preparation

A TCM injection sample was taken from the ampoule and filtered through a 0.22 µm membrane filter for injection.

10 mL of n-butyl alcohol saturated with water was added to 2 g of ophiopogonis and stored overnight. Nitrogen was used to remove some of the solvent to obtain a volume of 5 mL. Prior to use, the sample was filtered through a 0.22 µm nylon membrane filter.

Chromatographic conditions	
Mobile phase:	A = water, B = acetonitrile
Flow rate:	0.6 mL/min
Gradient:	20% B; 0–3 min, 20% to 40% B; 3–8 min, 100% B
Column temperature:	55 °C
DAD detector:	254 nm, 202 nm; 360/100 nm
Peak width:	0.006 min (40 HZ)
Slit:	4 nm
Injection:	0.5 µL
Autosampler injection with 5 s needle wash with acetonitrile	

Results and Discussion

First an Agilent ZORBAX RRHT SB C18 column (3.0 mm × 150 mm) was used for method optimization. Comparing the chromatograms acquired from different wavelengths (202 and 254 nm), it was determined that the 254 nm channel resulted in less peaks. This showed that most of the components have no UV absorbance at 254 nm. Based on previous research[3], saponins only have absorbance at low wavelengths. So 202 nm was selected as the data collecting channel for the remainder of the sample. A mass spectrometer is best as an additional detector for further confirmation if no other detector has been specified for qualitative analysis[5,6].

The structure of ginsenoside Re and Rg1 have very similar retention behavior in reverse phase chromatography. It is critical, therefore, that the method is able to separate them. In this study, separation of ginsenoside Re and Rg1 was achieved but with some tailing peaks. When the ZORBAX RRHT Eclipse Plus C18 column (3.0 mm × 150 mm) was tested for the separation, good peak shapes were achieved. Good separation was not achieved for some difficult saponin pairs such as ginsenoside Rg1 and Re. In order to achieve good peak shape but also satisfactory separation, two columns were connected forming a hybrid column. The results of the hybrid column compared to the two separate columns are listed in Table 1. From these results, it can be seen that no distinct efficiency was lost when the hybrid column was used.

	Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm				Agilent ZORBAX RRHD Eclipse Plus C18, 2.1 mm × 100 mm				Agilent ZORBAX RRHD SB C18, 2.1 mm × 50 mm connected with ZORBAX Eclipse RRHD Plus C18, 2.1 mm × 100 mm			
	Flow rate				Flow rate				Flow rate			
	0.2 mL/min		0.4 mL/min		0.2 mL/min		0.4 mL/min		0.2 mL/min		0.4 mL/min	
Pressure (bars)	132		262		258		483		374		718	
	RT	Plates	RT	Plates	RT	Plates	RT	Plates	RT	Plates	RT	Plates
Peak 1	0.890	2799	0.444	2415	1.751	9223	0.890	8057	2.563	15053	1.306	14159
Peak 2	1.276	4291	0.635	3698	2.574	12513	1.306	11776	3.753	19978	1.911	18676
Peak 3	2.725	7313	1.351	7870	5.711	16257	2.877	19624	8.249	23649	4.242	28078
Peak 4	5.841	8400	2.902	9081	12.945	17612	6.540	21667	18.380	24747	9.505	29618

Isocratic sample:	p/n 01080-68704							
Peak 1:	Dimethyl phthalate	Peak 2:	Diethyl Phthalate	Peak 3:	Biphenly	Peak 4:	o-Terphenyl	
Conditions:	Isocratic: 65% acetonitrile in water		Data rate: 40 HZ,		Ambient temperature: (23 °C)			
Detection:	254, 202 nm, 360/100 nm, slit: 4 nm							
Injection volume:	1 µL							

Table 1
Column test comparing two separated columns to two connected columns.

Figure 1 shows the chromatogram of the Shenmai injection. These results show good separation and fast analysis. Compared to a previously developed method that requires 100 minutes, the method developed in this Application Note is quicker, requiring only 11 minutes. The "difficult" ginsenoside pairs Re and Rg1 also achieved good separation.

Ophiopogonis extract was analyzed under the same optimized conditions (Figure 2)[7]. This analysis proved that *ophiopogonis* extracts contain compounds that elute later than the components of red ginseng. However, when *ophiopogonis* and red ginseng were combined through processing, the components changed and in the corresponding position of the Shenmai injection, some peaks got smaller and some peaks disappeared.

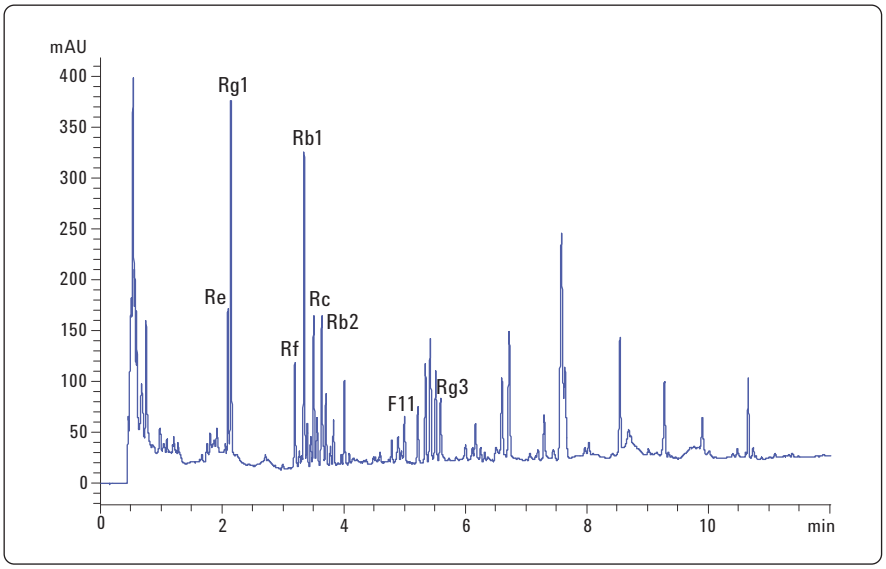

Figure 1
Chromatogram of Shenmai injection.

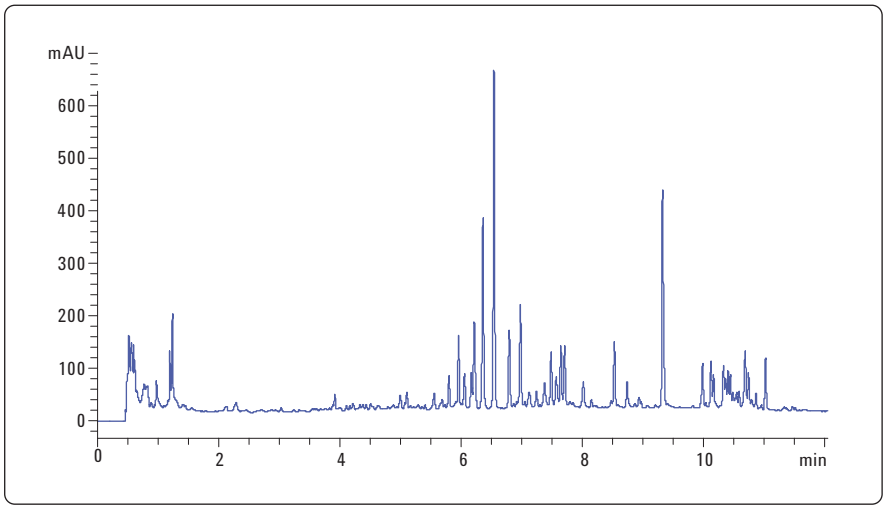

Figure 2
Chromatogram of extractions from *ophiopogonis*.

Five runs were performed to achieve good retention time and peak area (height) reproducibility (Table 2). The system is ideal for fast analysis of TCM samples with complex metrices. Figure 3 shows an overlaid chromatogram of five runs of Shenmai injection. The ultra high performance of the Agilent 1290 Infinity LC system is proven by the consistency of the results.

In these analyses the temperature was evaluated from 55 °C to ambient. The results showed that 55 °C can reduce the backpressure, produce faster analyses, and provide good peak separation.

Flow rate is another parameter evaluated in this study. The flow rate was set from 0.2 mL/min to 0.6 mL/min. The sub-2-µm particle size of the packing material has the advantage of retaining good performance even with higher flow rates. A flow rate of 0.6 mL/min was chosen to leverage fast speed and MS compatibility.

	RSD (%)		
Compounds	**Retention time**	**Peak height**	**Peak area**
Re	0.042	1.36	5.94*
Rg1	0.053	1.49	0.54
Rf	0.038	2.64	0.93
Rb1	0.047	1.29	0.64
Rc	0.045	2.47	0.37
Rb2	0.042	1.59	0.51
F11	0.026	0.96	1.22
Rg3	0.019	0.80	1.40

Table 2
Statistical results of repeatability.

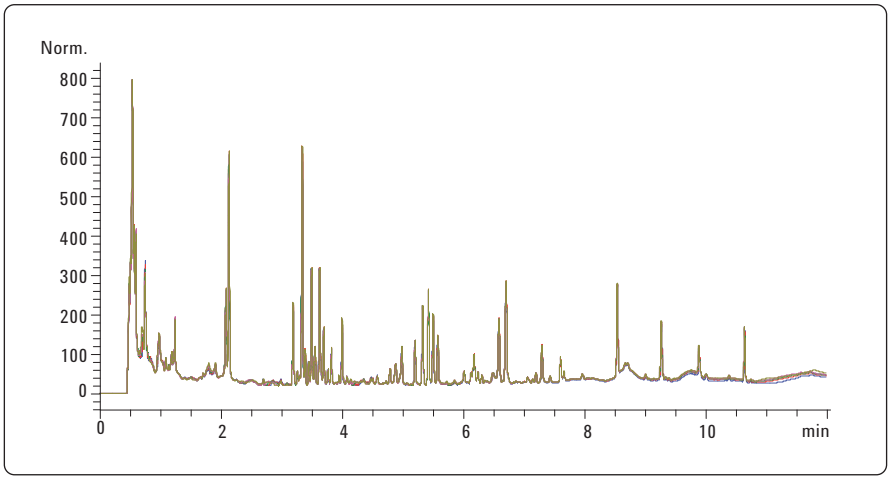

Figure 3
Overlaid chromatograms of five TCM injection sample.

Conclusion

A UHPLC method was established for the separation of Shenmai injection. This method is fast with high efficiency, obtaining both good separation and peak shape. This method can also be used for the analysis of red ginseng and ophiopogonis. This study showed that the connection of two columns did not decrease efficiencies through the capillary connection. The validation indicated that the developed method was sensitive, reliable and rapid. In addition, column temperature and flow rate were also tested to optimize these parameters. The method developed here can be used for high-throughput analysis when therapeutic effect research is in process. Further works will be done, based on the current separation method to obtain identification of additional components.

References

1.
Kunmei Ji, Jiajie Chen, Meng Li, Zhigang Liu, Lixin Xia, Chunbo Wang, Zhasngke Zhan, Xuli Wu, "Comments on serious anaphylaxis caused by nine Chinese herbal injections used to treat common colds and upper respiratory tract infections." Regulatory Toxicology and Pharmacology 55 (2009) 134–138. China

2.
Min Ye, Dean Guo, Guan Ye and Chenggang Huang, "Analysis of Homoisoflavonoids in Ophiopogon japonicus by HPLC-DAD-ESI-MSn," *J Am Soc Mass Spectrom* 2005, 16, 234-243

3.
Fuzzati N., "Analysis methods of ginsenosides." *J. Chrom.* B1-2, 812, 114–133, 2004.

4.
Fan Xiaohui, Wang Yi, Cheng Yiyu, "LC/MS fingerprinting of Shenmai injection: A novel approach to quality control of herbal medicines," *Journal of Pharmaceutical and Biomedical Analysis 40* (2006) 591-597

5.
Edgar Naegele, "Agilent MassHunter - Fast, computer-aided analysis of LC/ESI-TOF data from complex natural product extracts Part 2: Comparison of Agilent 6210 TOF data from different, biological origin with the Mass Profiler in MassHunter software," Agilent Technologies publication 5989-6076EN

6.
Edgar Naegele, Analysis of complex natural product extract from ginseng:

Part I: Structure elucidation of ginsenoside by rapid resolution LC-ESI TOF with accurate mass measurement Agilent Technologies publication 5989-4506EN

Part II: Structure elucidation of ginsenosides by high resolution ion trap LC/MS, Agilent Technologies publication 5989-4705EN

Part III: Species of differentiation of ginseng plants and authentication of ginseng products by LC/MS, Agilent Technologies publication 5989-5493EN

7.
Bai Jing, Yu Zhiguo, "HPLC fingerprint analysis of Radix ophiopogonis Chinese Traditional Patent Medicine," 29 (1), 2007, 12–13

www.agilent.com/chem/tcm

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