



Liquid Chromatography

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HPLC Analysis of Common Ginsenosides Found in Ginseng Using a Method Based on the Chinese Pharmacopoeia

Introduction

The term ginseng can refer to several natural products and herbal remedies usually produced from the plant, panax ginseng. Panax ginseng is considered a traditional Chinese medicine (TCM) as it has a reported wide range of medicinal properties such as antioxidant effects and promotion of immune function.¹ The chemistry of ginseng is still not fully understood and more research is required to understand its functions as it becomes increasingly popular in western culture. The main active components of ginseng are ginsenosides of which there are over 100 in various forms of panax ginseng. Ginsenosides are named as Ginsenoside Rx, with x representing the movement on a thin layer chromatography plate, x represents a letter from a-h with a being the most polar and h being the least.²

This application brief describes the use of an Epic™ C18 to analyse three ginsenosides using a method based on the Chinese Pharmacopoeia (ChP)³.

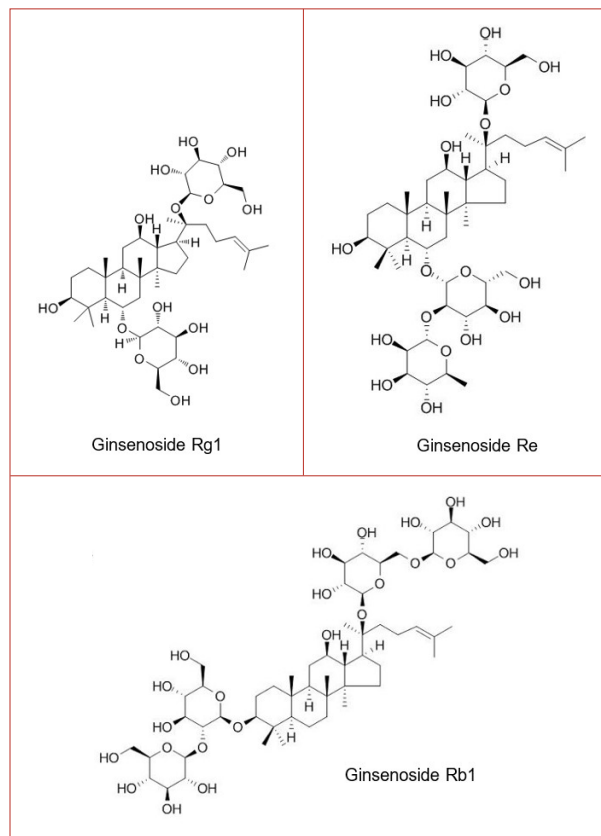


Figure 1. Structure of Ginsenosides Rg1⁴, Re⁵ and Rb1⁶.

Experimental Conditions

Method Parameters

All HPLC method parameters are shown in Table 1.

Table 1. HPLC method parameters.

Instrument	PerkinElmer Flexar™ with UV/VIS Detector		
Column	Epic C18 150 x 4.6 mm, 5 µm (P/N: 135291-EC18)		
Mobile Phase	A: Acetonitrile	B: Water	
Gradient Profile	Time	A%	B%
	0	19	81
	35	19	81
	55	29	71
	70	29	71
	100	40	60
101	19	81	
Flow Rate	1.0 mL/min		
Temperature	Ambient		
Wavelength	203 nm		
Injection Volume	10 µL		
Analyte	Ginsenosides Rg1, Re and Rb1		

Solvents and Samples

All solutions were filtered using a 0.45 µm, 17 mm nylon filter, P/N: 02542880.

A standard containing all three ginsenosides, each at 0.2 mg/mL, was made up in 100% methanol.

Results and Discussion

The ChP monograph specifies that a C18 phase be used. The Epic C18 (150 x 4.6 mm, 5 µm) column (P/N: 135291-EC18) meets this requirement and facilitates a repeatable separation with an RSD <1 % for all peaks, Table 2. The repeatability of the Epic C18 column is further demonstrated by the overlay of three replicate injections, Figure 3.

Two closely eluting peaks, ginsenosides Rg1 and Re, have been clearly and effectively separated with a resolution of 2.44, as a result of the superior base deactivation and high-density bonding technology of the Epic C18.

Table 2. System suitability results summary using Epic C18.

Peak Area RSD (%)*			Resolution (between peaks 1 and 2)
Ginsenoside Rg1	Ginsenoside Re	Ginsenoside Rb1	
0.48	0.98	0.66	2.44

*RSD calculated from three successive injections.

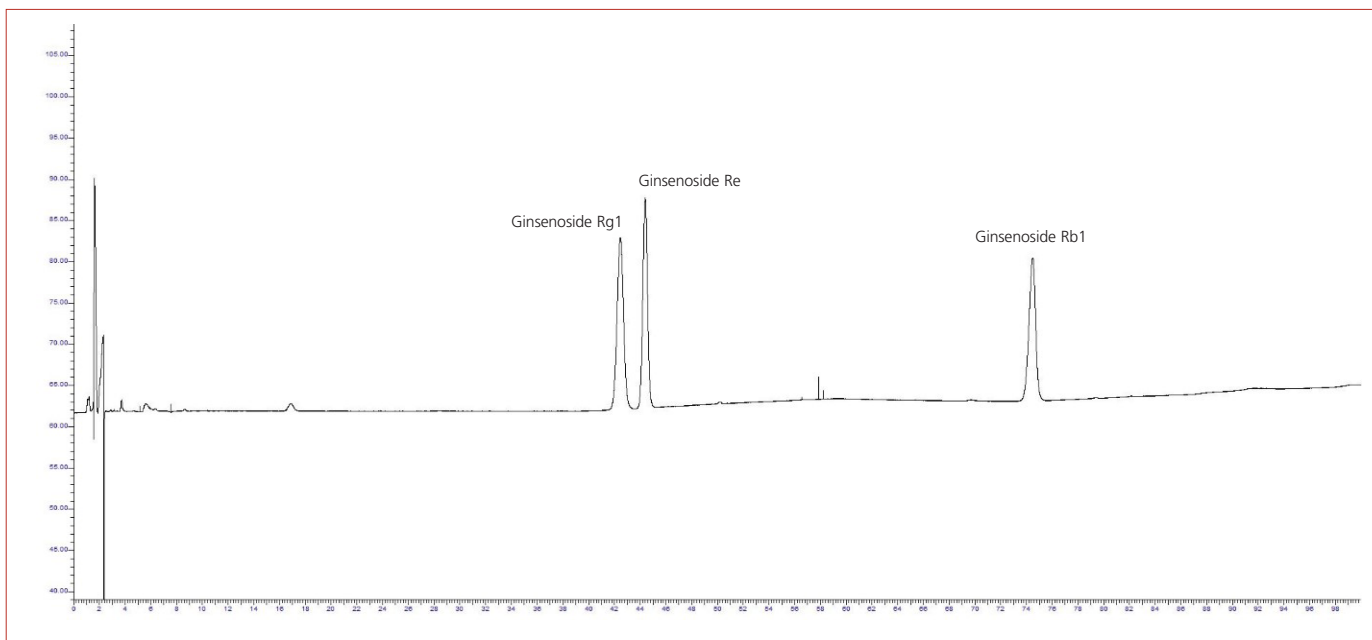


Figure 2. Analysis of common ginsenosides on an Epic C18 150 x 4.6 mm, 5 µm.

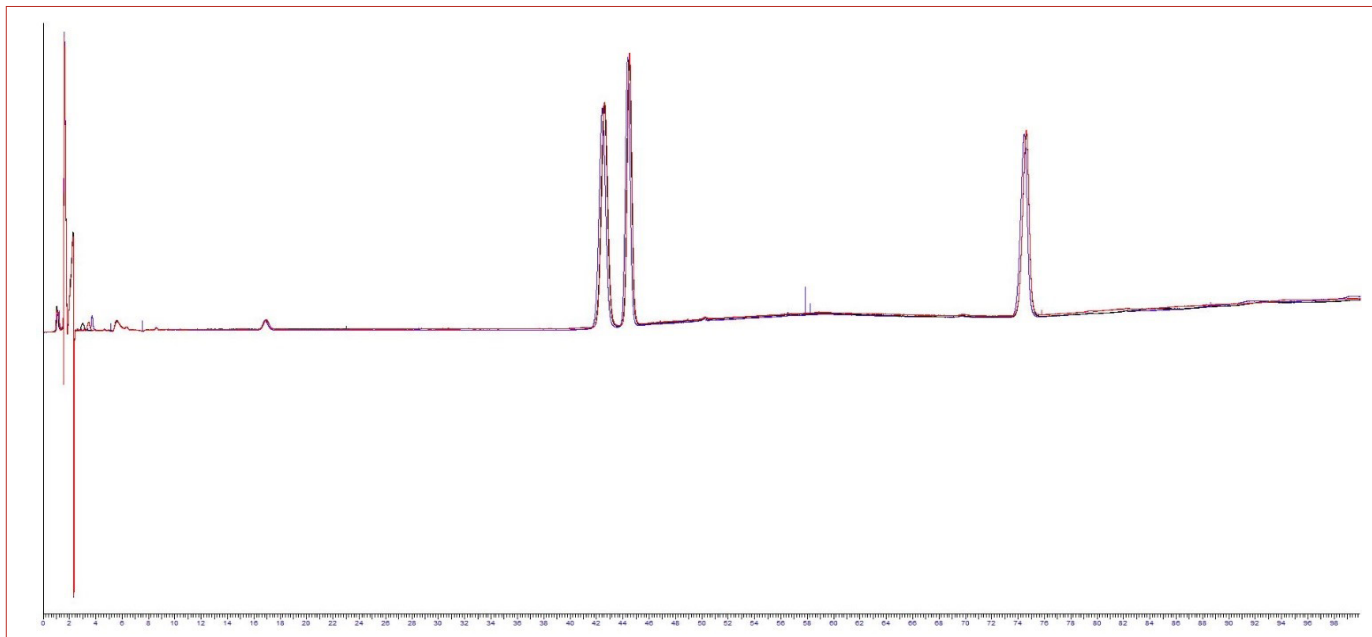


Figure 3. Overlay of three successive replicates of ginsenosides Rg1, Re and Rb1.

Conclusion

- The Epic C18 phase offers a repeatable separation of common ginsenosides with each compound having an RSD under 1%.
- The Epic C18 offers good resolution between the closely eluting peaks of ginsenosides Rg1 and Re, with a resolution of 2.44.
- Further optimisation could be made to the gradient method to reduce analysis time.

References

1. Benzie and S. Wachtel-Galor, Herbal medicine, CRC Press, Boca Raton, 2nd edn., 2011.
2. J. Kim, Y. Yi, M. Kim and J. Cho, Journal of Ginseng Research, 2017, 41, 435-443.
3. Pharmacopeia of the Peoples Republic of China, 10th edition, volume 1, 2015
4. ChemSRC website, https://www.chemsrc.com/en/cas/22427-39-0_314530.html, (accessed 04/03/2021)
5. Chemfaces website, <http://www.chemfaces.com/natural/Ginsenoside-Re-CFN99974.html>, (accessed 04/03/2021)
6. Chemfaces website, <http://www.chemfaces.com/natural/Ginsenoside-Rb1-CFN99964.html>, (accessed 04/03/2021)

Consumables

Component	Description	Part Number
Column	Epic C18 (150 x 4.6 mm, 5 µm)	135291-EC18
HPLC Vials	2 mL Amber 9 mm Screw Top Vial with Write-on Patch and Fill Lines (100/pack)	N9307802
HPLC Vial Caps	9 mm Screw Top Blue (polypropylene) Cap with PTFE/Silicone pre-slit Septa (100/pack)	N9306203
Syringes	Syringe 1 mL BD Luer-Lok Disposable, (100/pack)	02542890
Syringe Filters	0.45 µm Nylon Filter, 17 mm Diameter	02542880
PEEK Fittings	Finger tight for 1/16" OD PEEK tubing	09920513
Stainless Steel Fittings	OptiTech Reusable Nut/Ferrule for UHPLC	N9306301