APPLICATION NOTE

UHPLC

Author

Njies Pedjie

PerkinElmer, Inc.
Shelton, CT 06484 USA

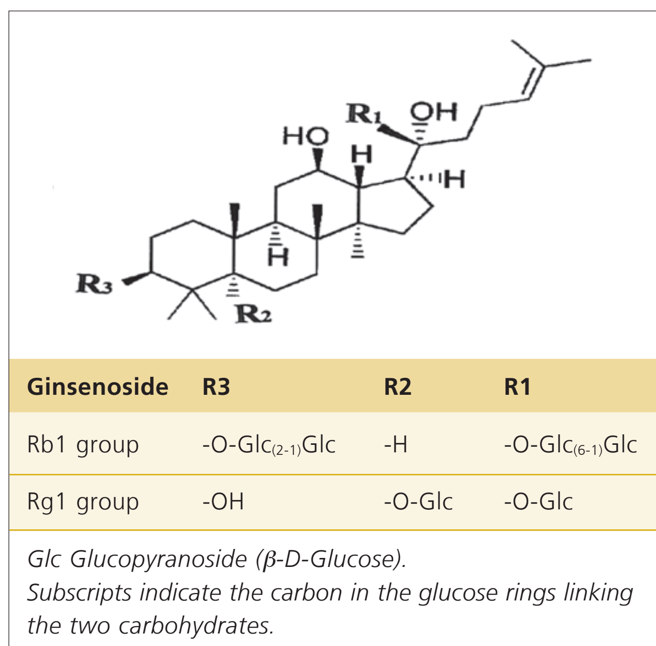
Analysis of Ginsenosides in Ginseng Root with the PerkinElmer Flexar FX-15 System Equipped with a PDA Detector

Introduction

The root of the panax genus plant (also called Ginseng) has been used as an herbal medicine in Asia for over two thousand years for its purported various health benefits, including (but not limited to), antioxidant, anticarcinogenic, anti-inflammatory, antihypertensive and anti-diabetic. The pharmacologically active compounds behind the claims of ginseng's efficacy are ginsenosides; their underlying mechanism of action although not entirely elucidated appears to be similar to that of steroid hormones. There are a number of ginseng species, and each has its own set of ginsenosides. In fact, more than forty different ginsenosides have been identified. Ginsenosides are a diverse group of steroidal saponins with a four ring-like steroid structure with sugar moieties (Figure 1); they are found exclusively in ginseng plants and are in higher concentration in their roots. There are two main groups of ginsenosides: the panaxadiol group or Rb1 group that includes Rb1, Rb2, Rc, Rd, Rg3, Rh2, and Rh3; and the panaxatriol group or Rg1 group that includes Rg1, Re, Rf, Rg2 and Rh1.

PerkinElmer
For the Better

Qualitative and quantitative analytical techniques for the analysis of ginsenosides are in demand to ensure quality control in ginseng root processing, as well as for the study of their metabolism and bioavailability. This application note presents a robust liquid chromatography method to simultaneously test seven ginsenosides. Method conditions and performance data including precision, accuracy and linearity are presented. The method is applied to a panax ginseng (Korean Ginseng) root capsules and the types of ginsenosides are confirmed.



Ginsenoside	R3	R2	R1
Rb1 group	-O-$Glc_{(2\text{-}1)}$Glc	-H	-O-$Glc_{(6\text{-}1)}$Glc
Rg1 group	-OH	-O-Glc	-O-Glc

Glc Glucopyranoside (β-D-Glucose).
Subscripts indicate the carbon in the glucose rings linking the two carbohydrates.

Figure 1. Molecular structure of ginsenosides.

Experimental

Seven stock standard solutions of each ginsenoside at 1 mg/mL concentration were prepared by dilution with 70:30 methanol/water (diluent), followed by one minute vortex. A working standard of 0.14 mg/mL was prepared by mixing together 0.5 mL of each of the stock solution.

Precision was evaluated with eight injections of the working standard. Linearity was determined across a range of 7 µg/mL to 140 µg/mL. To assess the accuracy of the method, purified water was spiked with the working standard to obtain a solution with 7 µg/mL ginsenosides. About 3 g of a panax ginseng powder from a popular brand capsules was transferred into 50 mL volumetric flask, 30 mL of diluent was added followed by about a minute vortexing and 30 min. sonication. The solution was then centrifuged at 5000 RPM for 10 min. and the supernatant was collected and set aside. 15 mL of diluent was added in the remaining precipitate followed by vortexing, sonication and centrifugation similar to that described above. This latter supernatant was collected and added to the first collected supernatant in a 50 mL volumetric flask; the solution was brought volume with diluent, mixed well and filtered with a 0.2 µm nylon membrane prior to testing.

A PerkinElmer® Flexar™ FX-15 UHPLC system fitted with a Flexar FX PDA photodiode array detector served as a platform for this experiment. The separation was achieved using a PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) column.

Table 1. **Detailed UHPLC system and chromatographic conditions.**

Autosampler:	Flexar FX UHPLC
Setting:	50 µL loop and 15 µL needle volume, partial loop mode
	350 µL mixer volume; injector wash and carrier: water
Injection:	2 µL
PDA Detector:	Scanned from 190-400 nm, recording setting 203 nm
UHPLC Column:	PerkinElmer Brownlee SPP C-18, 50 x 2.1 mm, 2.7 µm (superficially porous particles) at 45 °C, Part No. N9308402
Mobile Phase:	A: water
	B: acetonitrile
	(see gradient table below)
	3 minutes equilibration after each run
	(HPLC grade solvent and ACS grade reagent)
Sampling Rate:	5 pt/s
Software:	Chromera® Version 3.0

Time (min)	Flow rate (mL/min)	B %	Curve
2.5	0.4	30-35	1
3.5	0.4	35-50	1

Results and Discussion

The optimal flow rate of this method was determined to be 0.4 mL/min. at 45 °C, the pressure stabilized around 5150 PSI (355 bar) and all the peaks eluted within six minutes. A representative chromatogram of the standards solution and the Korean ginseng tested are in Figure 2 and 3. Excellent method performance was achieved: the linearity of the analysis had a R-squared of not less than 0.997 for each ginsenoside and a precision (relative standard deviation %RSD) with values ranging from 0.6% to1.2%. The spiked purified water tested had an average recovery of 99.9% with values ranging from 91.2% to108.0% (Figure 4). Details of the method performance and results of the panax ginseng and spiked sample tested are presented in Table 2.

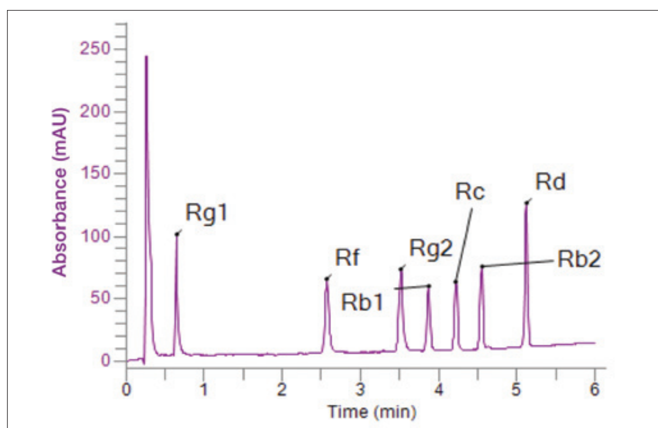


Figure 2. Chromatogram from the analysis of a standard.

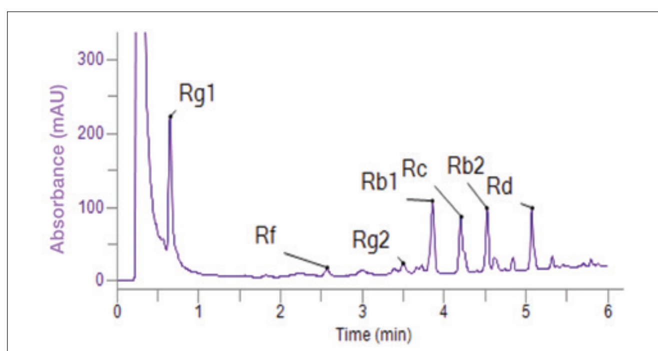


Figure 3. Chromatogram from the analyses of panax ginseng.

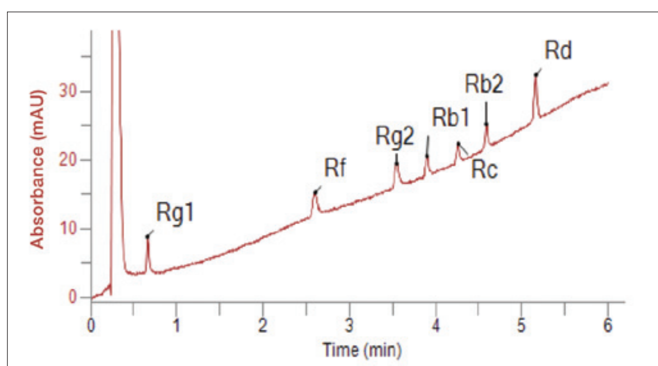


Figure 4. Chromatogram from the analyses of 7 ppm ginsenosides in water.

Table 2. **Precision, linearity, accuracy and samples.**

Com-pound	%RSD n = 8	r^2	Range (µg/mL)	Korean Ginseng (mg/g)	7 ppm Spiked Water
Rg1	0.9	0.9997	7 - 140	13	97.5
Rf	0.6	0.9971	7 - 140	1	91.2
Rg2	1.2	0.9983	7 - 140	1	98.7
Rb1	1.1	1	7 - 140	10	102.1
Rc	1.2	0.9994	7 - 140	10	100.3
Rb2	1.0	0.9996	7 - 140	7	101.4
Rd	1.2	0.9997	7 - 140	4	108.0
Avg/Tot.	**1.0/NA**	**0.9988/NA**	**NA**	**NA/46**	**99.9/NA**

NA = Not Applicable

Conclusion

The seven ginsenosides were well resolved within six minutes. The method was shown to be linear with R-squared ≥ 0.997, precise with %RSD ≤1.2 and accurate with a recovery averaging 99.9%. The Korean ginseng capsule tested has 46 mg/g of ginsenosides. PerkinElmer's Flexar FX PDA detector provides rugged and accurate detection over a range of 190 nm to 700 nm, encompassing UV and visible wavelengths. PerkinElmer's Chromera software offers many data acquisition and processing features: spectral library creation, and peak purity, spectra 3-D and contour maps, which are powerful tools that give insight to the information content of a 3-D photodiode array chromatogram. The spectra library search function allowed the storage of standard peaks spectra that could later be used for peak identification confirmation in the sample.

References

1. Rebecca M. Corbit, Jorge F.S. Ferreira, Stephen D. Ebbs, and Laura L. Murphy. Simplified Extraction of Ginsenosides from American Ginseng for High-Performance Liquid Chromatography-Ultraviolet Analysis J. Agric. Food Chem. **2005,** 53, 9867-9873 9867.

2. Attele, A.S.; Wu, J.A.; Yuan, C.-S. Ginseng Pharmacology: Multiple Constituents and Multiple Actions. *Biochem. Pharmacol.* **1999,** 58, 1685-1693.

Note: This application note is subject to change without prior notice.

PerkinElmer, Inc.
940 Winter Street
Waltham, MA 02451 USA
P: (800) 762-4000 or
(+1) 203-925-4602
www.perkinelmer.com

PerkinElmer®

For a complete listing of our global offices, visit www.perkinelmer.com/ContactUs

Copyright ©2011, PerkinElmer, Inc. All rights reserved. PerkinElmer® is a registered trademark of PerkinElmer, Inc. All other trademarks are the property of their respective owners.

009836_01