Liu et al., Afr J Tradit Complement Altern Med. (2015) 12(2):12-16
http://dx.doi.org/10.4314/ajtcam.v12i2.3

RP-HPLC DETERMINATION OF GINSENOSIDES RG1 AND RB1 IN *PANAX GINSENG* FRUITS

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Abstract

Background: At present, there has been a lot of research at home and abroad on the roots, stems and leaves of *Panax Ginseng* as well as their extracts, but the fruits of *Panax Ginseng* have been relatively little studied. **Materials and Methods:** To establish a method for determination of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits. RP-HPLC method is adopted, column used is a ZOBAX SB-C_{18} column (4.6 mm × 250 mnl, 5 μm), mobile phase A is water, and B is acetonitrile, gradient elution conditions are: 0~20 min (A:B 20:80), 20~60 min (A:B 20~35: 80~65); and detection wavelength 203 nm.
Results: Ginsenosides Rgl and Rb1 have good linear relationships within the ranges of 1.04~10.40 μg and 0.50~5.00 μg, respectively, and r is 0.9998 and 0.9997; reproducibility and recovery of the method are both in line with requirements. **Conclusion:** The method established is simple, accurate and fast, which is suitable for the simultaneous determination of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits.

Keywords: ginsenoside Rg1; ginsenoside Rb1; HLPC; leukemia

Introduction

Panax Ginseng fruits are the ripe fruits of *Panax Ginseng* C. A. Mey in the family Araliaceae. *Panax Ginseng* fruit contains polysaccharides and ginsenosides; besides, its ginsenoside content is higher than that in the underground part of *Panax Ginseng* (Sun, 2011; Wang et al., 2004; Zhao et al., 1991; Yang et al., 2009). Modern medical studies have found that *Panax Ginseng* fruit has anti-cancer, anti-shock, myocardial protective, anti-aging and immunity enhancing effects (Wang et al., 2007; Lei at al., 2008; Huo, 1984; NISHI et al., 2004).

At present, there has been a lot of research at home and abroad on the roots, stems and leaves of *Panax Ginseng* as well as their extracts, but the fruits of *Panax Ginseng* have been relatively little studied. The 2010 edition of "Pharmacopoeia of the People's Republic of China" included the quality standards for *Panax Ginseng* and *Panax Ginseng* leaves (Chinese Pharmacopoeia Commission, 2010), but *Panax Ginseng* fruits have not been included; moreover, the determination method of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits has not been reported in the literature as well. This study aims to establish a method for the determination of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits, which can be used as a quality control indicator of *Panax Ginseng* fruits, and provides the experimental basis for better quality control of *Panax Ginseng* fruits and related products. Ginsenosides are the most important physiologically active constituents of *Panax Ginseng*, of which dammarane-type triterpenoid saponins have the most significant activity; determination of ginsenosides has become an important indicator for evaluation of *Panax Ginseng* and its products. Overground part of *Panax Ginseng* is an unignorable source of ginsenosides; *Panax Ginseng* fruit contains ginsenosides Rg1 and Rb1, and pharmacological experiments have shown that ginsenosides Rg1 and Rb1 have significant inhibitory effects on leukemia cancer cell proliferation (Wang et al., 2009; MATSUDA et al., 2005). In this study, RP-HPLC method is adopted to determine two kinds of ginsenosides in *Panax Ginseng* fruits. The method has good resolution, and is accurate and fast, which is suitable for the determination of ginsenoside content in *Panax Ginseng* fruits.

Instruments and Reagents

HPLC system (Agilent 1260, USA); ultrasonic extractor (CSQX-263, Shanghai Kelaimente Instrument Co., Ltd.); ginsenosides Rg1 and Rb1 reference substances (National Institute for the Control of Pharmaceutical and Biological Products, batch numbers: 20141234504R and 20140458215HS, respectively); HPLC grade methanol; ultrapure water. *Panax Ginseng* fruits were purchased from Tongrentang Pharmacy (batch numbers: 20140704, 20140705, 20140706), which were identified by Professor Wang Min of Capital Medical University as the ripe fruits of Araliaceae plant *Panax Ginseng* C. A. Mey.

Methods

Chromatographic conditions and system suitability test column: ZOBAX SB-C_{18} column (4.6 mm × 250 mm, 5 μm); mobile phase: ultrapure water (A); acetonitrile (B); flow rate: 1.0 mL/min. Gradient elution conditions: 0~20 min (A:B 20:80), 20~60min (A:B 20~35:80~65); detection wavelength: 203 nm; column temperature: 30□; injection volume: 10 μL per test. Samples were taken, processed as per the test solution preparation method, and then subjected to system suitability test. The results showed that the peak resolutions of ginsenosides Rg1 and Rb1 with other components met the requirements, and peak shapes were symmetrical, chromatograms are shown in Figure 1.

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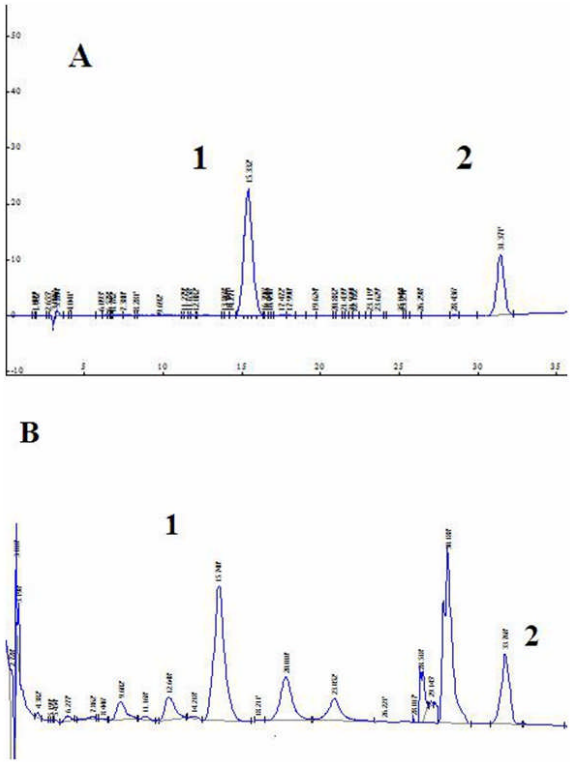

A Mixed reference substance B Test substance
1 Ginsenoside Rg1 2 Ginsenoside Rb1
Figure 1a: HPLC chromatograms

Preparation of solutions

(1) Reference solutions: appropriate amount of ginsenosides Rg1 and Rb1 reference substances were accurately weighed, and prepared into 0.52 mg/ml and 0.25 mg/mL reference stock solutions, respectively, with methanol.
(2) Test solution: 50 mg of *Panax Ginseng* fruit powder (passed through a 80-mesh sieve) was accurately weighed, placed in a 25 mL volumetric flask, added with appropriate amount of methanol, shaken well, and then ultrasonicated for 10 min (ultrasonic frequency 40 Hz, power 60 W) for dissolution. After being cooled to room temperature, the resulting solution was added with methanol precisely to the mark, stoppered, shaken well, and filtered through a microporous membrane (0.45 μm) to give the test solution.
(3) Investigation of linear relationship: reference stock solutions were precisely drawn, and prepared into a series of standard solutions, 2, 4, 8, 12, 16 and 20 μL of which were injected into the chromatograph, and determined according to the above chromatographic conditions. Peak areas were recorded, and linear equations were obtained with the peak area (A) as ordinate and the concentration (C, μg) as abscissa, the results showed good linear relationships of each constituents, see Table 2 and Figures 1 and 2.

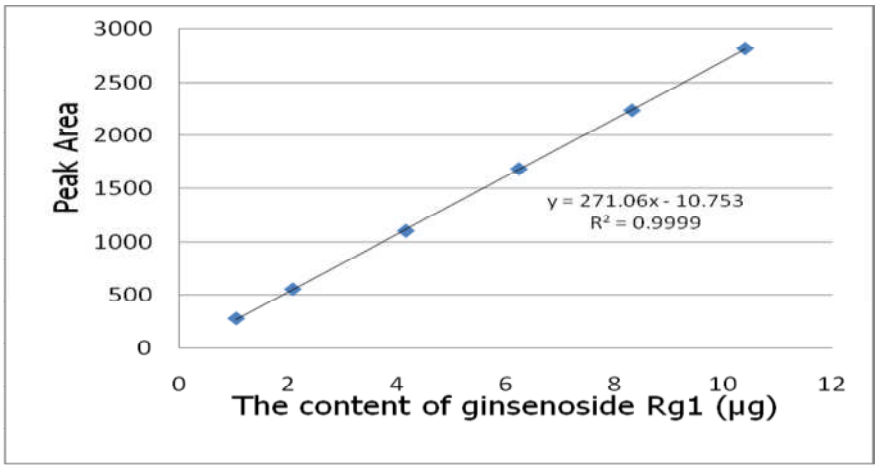

Figure 1b:. Linear relationship of ginsenoside Rg1

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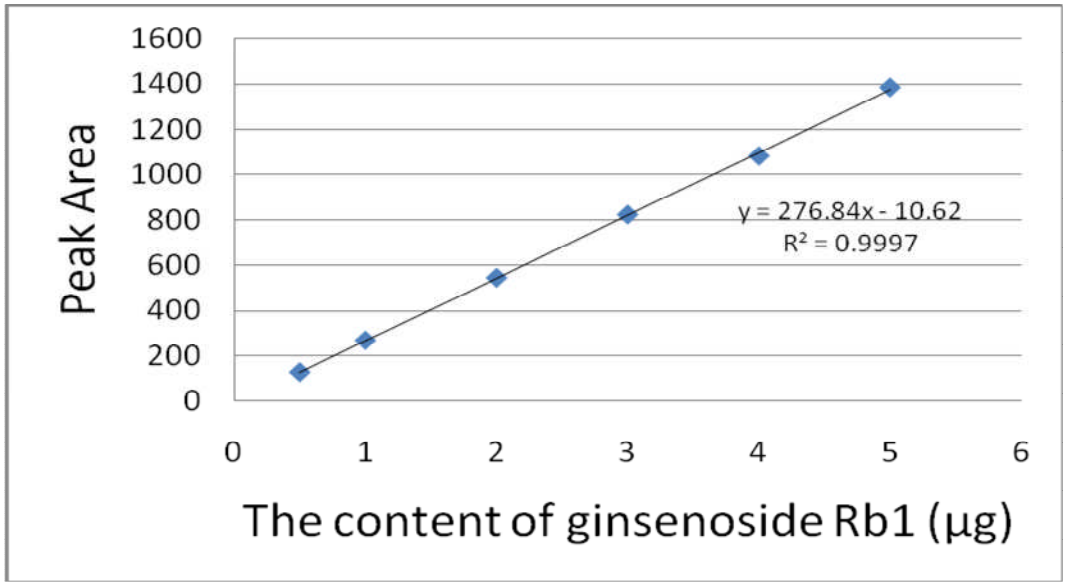

Figure 2: Linear relationship of ginsenoside Rb1

Table 1: Concentration and peak area of ginsenosides Rg1 and Rb1

No.	Rg1		Rb1	
	Concentration (μg)	A	Concentration (μg)	A
1	1.04	278.566	0.5	127.523
2	2.08	555.658	1	267.659
3	4.16	1101.542	2	545.236
4	6.24	1684.635	3	821.616
5	8.32	2238.891	4	1082.369
6	10.4	2815.324	5	1382.966

Table 2: Linear scheme of two constituents (n = 6)

Constituent	Linear equation	r	Linear range (μg)
Ginsenoside Rg1	y = 271.06x - 10.753	0.9999	1.04~10.40
Ginsenoside Rb1	y = 276.84x - 10.62	0.9997	0.50~5.00

Results

Precision test

10 μL of aliquots of the same test solution under "Test solution" were precisely drawn, and injected five times continuously. The results showed that RSDs of ginsenosides Rg1 and Rb1 were 0.52% and 0.98%, respectively, indicating good precision of the instrument.

Stability test

Test solution was freshly prepared as per the method in "Test solution", placed at room temperature, and injected at 0, 2, 4, 6, 8 and 24 h, respectively, for analysis according to the above chromatographic conditions. Peak areas of the two constituents were measured, and RSDs of ginsenosides Rg1 and Rb1 were obtained to be 1.25% and 1.87% (n = 6), respectively, the results indicated good stability of the two constituents in test solutions within 24 h.

Reproducibility test

Panax Ginseng fruit powder was accurately weighed, and prepared into five aliquots of parallel test solutions as per the method in "Test solution", and the contents of ginsenosides Rg1 and Rb1 were determined according to the above chromatographic conditions. Average contents of the two constituents were obtained to be 1.76% and 0.98%, with RSDs of 2.01% and 2.59% (n = 5), respectively, the results indicated good reproducibility of the method.

Recovery test

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0.05 g of six aliquots of *Panax Ginseng* fruit powder of known content with the same batch number were accurately weighed, added precisely with 2 mL of ginsenosides Rg1 and Rb1 reference solutions, respectively, and operated as per the test solution preparation and sample determination methods, followed by calculation of recovery. The results are shown in Table 3, which indicates good recovery of each constituent.

Table 3: Recovery test results

Constituent	No.	Sample amount (mg)	Addition amount (mg)	Measured amount (mg)	Recovery (%)	Mean (%)	RSD (%)
Ginsenoside Rg1	1	0.880	1.04	1.895	97.60	98.54	1.51
	2	0.880	1.04	1.925	100.48		
	3	0.880	1.04	1.909	98.94		
	4	0.880	1.04	1.890	97.12		
	5	0.880	1.04	1.920	100.00		
	6	0.880	1.04	1.890	97.12		
Ginsenoside Rb1	1	0.490	0.50	0.990	1.00	97.67	3.97
	2	0.490	0.50	0.980	0.98		
	3	0.490	0.50	0.960	0.94		
	4	0.490	0.50	0.990	1.00		
	5	0.490	0.50	1.000	1.02		
	6	0.4900	0.50	0.950	0.92		

Content determination

Panax Ginseng fruit powders with different batch numbers were taken, prepared into test solutions as per the method in "Test solution", and quantitatively determined in three parallels according to the above chromatographic conditions, followed by calculation of content of the two constituents. The results are shown in Table 4.

Table 4: Content determination results (n = 3)

Batch No.	Ginsenoside Rg1		Ginsenoside Rb1	
	x（%）	RSD（%）	x（%）	RSD（%）
20140704	1.76	1.32	0.98	1.52
20140705	1.78	0.64	0.96	1.23
20140706	1.75	1.23	0.96	1.43

Discussion

Notoginsenosides are special constituents of Araliaceae plants, of which representative constituents, ginsenosides Rg1 and Rb1, have gained widespread attention in recent years for their anti-cancer effects (Park et al., 2008; Jang et al., 2012; Fan et al., 1995; Li et al., 2014; Song et al., 2013). Research has reported that ginsenosides Rg1 and Rb1 have inhibitory effects on leukemia K562 and HL-60 cells (Wang et al., 2009; MATSUDA et al., 2005); although their anti-cancer mechanisms are still unclear, they have prompted us the possibility of leukemia treatment by ginsenosides Rg1 and Rb1-containing drugs. *Panax Ginseng* fruits, containing ginsenosides Rg1 and Rb1, are from the underground part of *Panax Ginseng*, which is undoubtedly a potential treatment of leukemia. So we studied the method for quantitative determination of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits.

The study establishes a RP-HPLC method for determination of ginsenosides Rg1 and Rb1 in *Panax Ginseng* fruits. Column temperature and flow rate had greater impacts on the isolation effect, so after repeated tests, column temperature was optimized as 30□, and flow rate as 1.0 mL/min. Determination results of single ginsenoside content in *Panax Ginseng* fruits revealed that ginsenosides Rg1 and Rb1 had higher contents than other ginsenosides in *Panax Ginseng* fruits. But compared with other parts of *Panax Ginsen*g, the contents of ginseng ginsenosides Rg1 and Rb1 were relatively low, without advantages; nevertheless, it does not affect the use of *Panax Ginseng* fruits as a source of novel anti-leukemia drugs. *Panax Ginseng* fruits are less studied than other parts of *Panax Ginsen*g; if *Panax Ginseng* fruits can be developed into new drugs, it will be a good utilization of *Panax Ginseng* fruit resources. The method established herein is accurate, fast, reproducible and highly sensitive, which can be used as *Panax Ginseng* fruit determination method, and provides the basis for the development of quality standards for *Panax Ginseng* fruit and its preparations.

The research is about the determination of Ginsenoside Rg1 and Ginsenoside Rb1 in *Panax Ginseng* fruit. Because of this method is simultaneous determination and conditions are more difficult to control, so the discovery of the method is difficulty. The research is also insufficient. The recovery of Rb1 RSD is 3.97, higher than normal. In this study, the pharmacological activity is lack. We will make sure the role of *Panax Ginseng* fruit extract and ginsenoside Rg1 and ginsenoside Rb1 on leukemia, and expound its mechanism of action in the next step.

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