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Quantitative determination of six major triterpenoids in *Ganoderma lucidum* and related species by high performance liquid chromatography

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Abstract

A reversed-phase liquid chromatographic method was developed for the quantitative determination of six triterpenoids, namely ganoderic acids C_2, B, AM_1, K, H and D in *Ganoderma lucidum* and its related species. Samples were extracted with chloroform in ultrasonic bath. The optimal conditions of separation and detection were achieved on an Agilent Zorbax SB-C_{18} column (250 mm × 4.6 mm, 5 μm), with a linear gradient of acetonitrile and 0.03% aqueous phosphoric acid (v/v), at a flow rate of 1.0 ml/min, detected at 252 nm. All calibration curves showed good linearity ($r^2 > 0.999$) within test ranges. The relative deviation of this method was less than 2% for intra- and inter-day assays, and the percentage recovery of the method was 93–103%, with relative standard deviation (R.S.D.) less than 5%. The current assay method was applied to quantitative determination of constituents of triterpenoids in 36 different samples of *G. lucidum* and its related species. The results indicated that the developed method could be readily utilized as a quality control method for *G. lucidum* and related species.

Keywords: *Ganoderma lucidum*; Triterpenoids; Reversed-phase high performance liquid chromatography

1. Introduction

Ganoderma lucidum (Leyss. ex Fr.) Karst (Polyporaceae), commonly called "Lingzhi" in China, is a well-known crude drug which has long been used in traditional Chinese medicine for the promotion of longevity and maintenance of vitality. In the 16th century, Lingzhi was cited in *Compendium of Materia Medica* (compiled by Li Shi-Zhen in Ming Dynasty) for enhancing "vital energy", increasing "intellectual capacity" and promoting "longevity", and this "mushroom of longevity" has been deemed as the sacred herb in China. Nowadays, it is still widely prescribed by traditional Chinese medical doctors for the treatment of debility and weakness, insomnia, hepatitis, cardiovascular diseases, cancer, etc. [1–4].

During the past two decades, more than 130 triterpenoids (including ganoderic acid derivatives) have been isolated from the fruiting bodies, cultured mycelia and spores of the *Ganoderma* [5,6]. Triterpenoids have received considerable attention owing to their conspicuous pharmacological activities. Some of these compounds showed anti-HIV-1 (ganoderic acids A, B, H, C_1) [7,8], antihistamine (ganoderic acids C_2, D) [9], antinociceptive (ganoderic acids A, B) [10], anticholesterol (ganoderic acids B, C_2) [11], and inhibitory activity of angiotensin converting enzyme (ganoderic acids K, F, S) [12]. Hence, the triterpenoids could be considered as the 'marker compounds' for the chemical evaluation or standardization of *G. lucidum*.

Owing to its satisfactory clinical effect, *Ganoderma* has been widely used as the major component of healthy foods and drugs for the time being in China. Therefore, development of quality control methods for *G. lucidum* and its related preparations is an essential issue for the effective clinical use of this medicinal herb. However, the previous studies on the quantitative analysis

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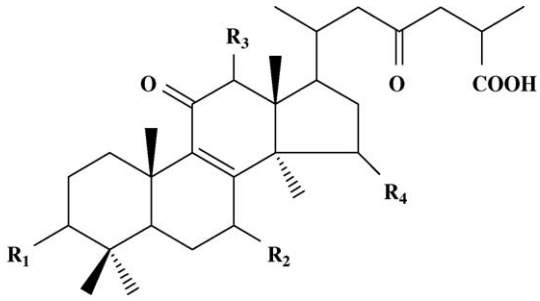

Ganoderic acid C_2 (1): R_1=R_2=β-OH, R_3=H, R_4=α-OH

Ganoderic acid B (2): R_1=R_2=β-OH, R_3=H, R_4=O

Ganoderic acid AM_1 (3): R_1=β-OH, R_2=R_4=O, R_3=H

Ganoderic acid K (4): R_1=R_2=β-OH, R_3= β-OAC, R_4=O

Ganoderic acid H (5): R_1=β-OH, R_2=R_4=O, R_3= β-OAC

Ganoderic acid D (6): R_1=R_4=O, R_2=β-OH, R_3=H

Fig. 1. Structures of six triterpenoids in *G. lucidum*.

of multiple triterpenoids in *G. lucidum* are insufficient [13–18] due to the difficulty to obtain the standard compounds. Furthermore, the authentication of commercial samples of *G. lucidum* was generally carried out by applying classical procedure performed by thin layer chromatography (TLC) [19]. In this study, a RP-HPLC method was developed for the simultaneous determination of six triterpenoids (shown in Fig. 1) with simple sample pretreatment methods. The developed method was successfully applied to the quantification of six major triterpenoids in 36 *Ganoderma* samples.

2. Experimental

2.1. Chemicals and materials

Acetonitrile and phosphoric acid were of HPLC grade (Burdick & Jackson, Honeywell International Inc., USA). HPLC grade water was prepared using a Milli-Q Water purification system (Millipore, MA, USA). The samples of *G. lucidum*, the major component of anticancer TCM formula SunRecome®, provided by Green Valley Pharmaceutical Co., China. And its related species were purchased from drug stores in different provinces of China.

The reference standards of triterpenoids **1**–**6** were isolated from the fruit bodies of *G. lucidum*. The dried fruit bodies (10 kg) were pulverized and extracted three times with 95% ethanol boiling in an immersion heater. The extract was concentrated under reduced pressure to yield residue (113 g), which was then suspended in hot H_2O and extracted with dichloromethane. The dichloromethane layer was concentrated to about 1/10 of its original volume and extracted with sat. aq. $NaHCO_3$ and the extract was acidified to pH 3–4 with 6 mol/l HCl at 0 °C. The resulting precipitate was dissolved in dichloromethane and then evaporated in vacuo to yield a residue (28 g), which was subjected to CC separation over silica gel (300 g) and eluted with a gradient of $CHCl_3$–MeOH (98:2–90:10, v/v) to afford 10 fractions (Fr. 1–10). Fr. 2 was further separated on silica gel column and eluted with $CHCl_3$–MeOH (95:5, v/v) to give three fractions (sFr. 1–3). Ganoderic acid D (17.3 mg) was deposited from sFr. 1 and recrystallized from acetone. sFr. 2 was further separated by preparative TLC (silica GF_{254}, 10–40 μm) with hexane–EtOAc–acetic acid (20:80:0.5) and two mixtures (M1 and M2) was obtained. M1 was separated by preparative HPLC and eluted with MeOH–0.5% HOAc (56:44, v/v) to give ganoderic H (38.9 mg) and ganoderic K (21.6 mg). M2 was subjected to preparative HPLC and eluted with MeOH–0.5% HOAc (53:47, v/v) to yielded ganoderic acid AM_1 (20.4 mg) and ganoderic acid B (24.1 mg). Ganoderic acid C_2 (41.8 mg) was deposited from sFr. 3 and recrystallized from MeOH. All these compounds were identified by direct comparison of their spectral data (UV, IR, NMR and MS) with those reported in the literature [12,20–24] and their purities were >98% determined by HPLC/UV analysis.

2.2. Apparatus and chromatographic conditions

An Agilent 1100 liquid chromatography system, equipped with a quaternary solvent deliver system, an autosampler and DAD detector, was used. A Zorbax SB-C_{18} column (250 mm × 4.6 mm, 5 μm) connected with a Zorbax SB-C_{18} guard column (20 mm × 4 mm, 5 μm) at temperature of 35 °C was applied for all analyses. Detection wavelength was set at 252 nm. The mobile phase consisted of (A) acetonitrile and (B) 0.03% apueous phosphoric acid (v/v) using a gradient elution of 30–32% A at 0–40 min, 32–40% A at 40–60 min. The flow rate was 1.0 ml/min and aliquots of 10 μl were injected.

2.3. Method validation

The method was validated for parameters such as linearity, precision, accuracy and stability following the International Conference on Harmonization (ICH) guideline [25].

2.3.1. Calibration curves

The mixture stock solution of ganoderic acids C_2 (**1**), B (**2**), AM_1 (**3**), K (**4**), H (**5**) and D (**6**) was prepared by dissolving the reference substances in methanol to final concentration of 510 μg/ml for ganoderic acid C_2, 555 μg/ml for ganoderic acid B, 540 μg/ml for ganoderic acid AM_1, 525 μg/ml for ganoderic acid K, 2010 μg/ml for ganoderic acid H and 675 μg/ml for ganoderic acid D, respectively, then diluted the mixture stock solution to appropriate concentration ranges for establishment of calibration curves. Linearity of each compound was determined with three injections for each concentration and plotted using linear regression of the mean peak area versus concentration.

2.3.2. Precision

The measurement of intra- and inter-day variability was utilized to determine the repeatability of the developed assay

method. The intra-day repeatability was examined on six individual sample solutions that were prepared from crude drug according to Section 2.6 within one day, and inter-day repeatability was determined for three independent days. The relative standard deviation (R.S.D.) was taken as a measure of repeatability.

2.3.3. Limits of detection (LOD) and quantification (LOQ)

The standard stock solutions were diluted with methanol to provide a series of solutions with the appropriate concentrations. The limit of detection and quantification under the chromatographic conditions were determined by measuring the signal-to-noise ratio for each compound by injecting a series of solutions until the S/N ratio 3 for LOD and 10 for LOQ.

2.3.4. Accuracy

Known quantities of the mixed standard solution were added into the known amounts of *G. lucidum* samples, and then the resultant samples were extracted and analyzed with the established HPLC method. The added standard solution was prepared in the concentration range of calibration curve with three different concentration levels (high, middle and low) and triplicate experiments were performed at each level. The percentage recoveries were evaluated by calculating the ratio of detected amount versus added amount.

2.3.5. Stability

Stability was tested with mixture stock solution and sample solution that were stored at 20–25 °C and analyzed every 12 h within 3 days. The relative standard deviation was taken as a measure of stability.

2.4. Optimization of extraction conditions

An orthogonal experiment was employed in order to optimize the extraction conditions. Three factors were involved: (A) solvent volume; (B) extraction times and (C) sonication time. The experimental factors, corresponding levels and orthogonal designs $L_9(3^4)$ were presented in Table 1. The total content of six triterpenoids in *Ganoderma* was used as a criterion for the selection of the optimal extraction conditions.

2.5. Optimization of chromatographic conditions

In order to obtain the chromatograms with better resolution of adjacent peaks within a short time, the column temperature, the flow rate of mobile phase and the compositions of mobile phase were optimized.

2.6. Sample preparation

G. lucidum was obtained from the cultivation base of Green Valley Pharmaceutical Group, China. A 2.0-g powder of dried samples was extracted with 40 ml $CHCl_3$ in an ultrasonic water bath for 20 min. This extraction was repeated twice. The extracted solution was mixed and filtrated through analytical filter paper and then the filtered solution was evaporated at 35 °C to dryness in vacuum. The dry extract was dissolved in 5 ml methanol and filtrated through a 0.45 μm membrane filter unit. Then 10 μl of each sample solution was analyzed by HPLC. The contents of the analytes were determined from the corresponding calibration curve.

Table 1
The results and analysis of orthogonal design for the optimization of extraction conditions

Run no.	A: solvent volume (ml)	B: extraction times (times)	C: sonication time (min)	T_6[a]
1	20	1	20	3070.2
2	20	2	40	2946.0
3	20	3	60	2172.1
4	30	1	60	3676.8
5	30	2	20	3894.7
6	30	3	40	3938.5
7	40	1	40	3842.7
8	40	2	60	4132.8
9	40	3	20	4532.5
K_1[b]	8188.3	10589.6	11497.4	
K_2	11510.0	10973.5	10727.2	
K_3	12508.0	10643.1	9981.7	
k_1[c]	2729.4	3529.9	3832.5	
k_2	3836.7	3657.8	3575.7	
k_3	4169.3	3547.7	3327.2	
Range	1439.9	128.0	505.2	
Optimized scheme	A_3	B_2	C_1	
Primary and secondary order	1	3	2	

[a] T_6 represents the total concentration of six triterpenoids in *G. lucidum* (μg/g).
[b] K represents the total values of the same level of the same factor.
[c] k represents the average values of the same level of the same factor.

3. Results and discussion

3.1. Optimization of extraction conditions

In order to obtain optimal extraction efficiency, variables involved in the procedure such as solvent and extraction method were optimized. Chloroform, methanol and chloroform–methanol solutions were tried as the extraction solvent. At last, chloroform was chosen as the extraction solvent since the triterpenoids could not only efficiently be extracted but also well resolved from background. Ultrasonic extraction was compared with refluxing. It was found that both extraction methods have the similar extraction efficiency but ultrasonic extraction was simpler, hence the ultrasonic bath extraction was chosen as the preferred method.

According to the statistic analysis shown in Table 1, the largest range of the three factors was 1439.9 of factor A; the smallest was 128.0 of factor B. This means that the factor A is the primary factor in the extract conditions of *G. lucidum*. The third level of factor A had the largest average value (k_3 is 4169.3) than the other two levels. It means that the third level is the best condition of factor A. Analogously the second level of factor B and the fist level of factor C were the best conditions. Therefore, the extraction conditions were optimized as the fol-

lowing: 2 g powder of dried samples was extracted twice with 40 ml chloroform in an ultrasonic water bath for 20 min.

3.2. Optimization of chromatographic conditions

According to the absorption maxima of six triterpenoids on the UV spectra with three-dimensional chromatograms of HPLC-DAD detection, the detection wavelength was performed at 252 nm. It was also suggested that the separation was improved when column temperature was increased to 35 °C and mobile phase was delivered with the flow rate of 1.0 ml/min.

Different mobile phase compositions and different ratio of phosphoric acid in water were also optimized. As a result, acetonitrile and water containing 0.03% phosphoric acid was chosen as the eluting solvent system since with it not only the desired separation but also acceptable tailing factor were acquired.

Chromatograms of standard mixture (A) and *Ganoderma* extracts of different origins and related species (B–D) were shown in Fig. 2. The chromatographic peaks of *Ganoderma* extracts were identified by comparing them with the retention time of each reference triterpenoid and UV spectrum recorded using the diode array detector. System suitability studies were carried out by using the standard mixture solution and evaluated by making five replicate injections. The system was deemed to be suitable for use because the tailing factor was less than 1.3, the resolution was greater than 1.5 and theoretical column plate number was more than 10,000 for each analyte.

3.3. Linearity, precision, accuracy and stability

Under the chromatographic conditions used in this study, all six calibration curves exhibited good linear regressions ($r^2 > 0.999$). Lack of fit test on regression between concentration and three detection values of the peak area exhibited non-significance and r square of regression equation exhibited significance via F-test at $\alpha = 0.05$ level for each calibration curves. The limits of detection were in the range of 0.22–0.30 μg/ml and the limits of quantification were in the range of 0.90–0.67 μg/ml for six triterpenoids. The results were presented in Table 2.

As the results shown in Tables 3 and 4, the developed analytical method was reproducible with good accuracy for all analytes. The intra- and inter-day variations were all less than 2% and the percentage recoveries were in the range of 93–103% with R.S.D less than 5% for six triterpenoids. According to the results shown in Table 5, stabilities of analytes in the mixture stock solution and sample solution were found to be stable within 3 days (R.S.D. < 3%).

3.4. Analysis of six triterpenoids in G. lucidum and its related species

As shown in Table 6, the developed RP-HPLC assay method was successfully applied for the determination of six triterpenoids in different *Ganoderma* samples. On the basis of these experiments, we concluded that the content and composition of

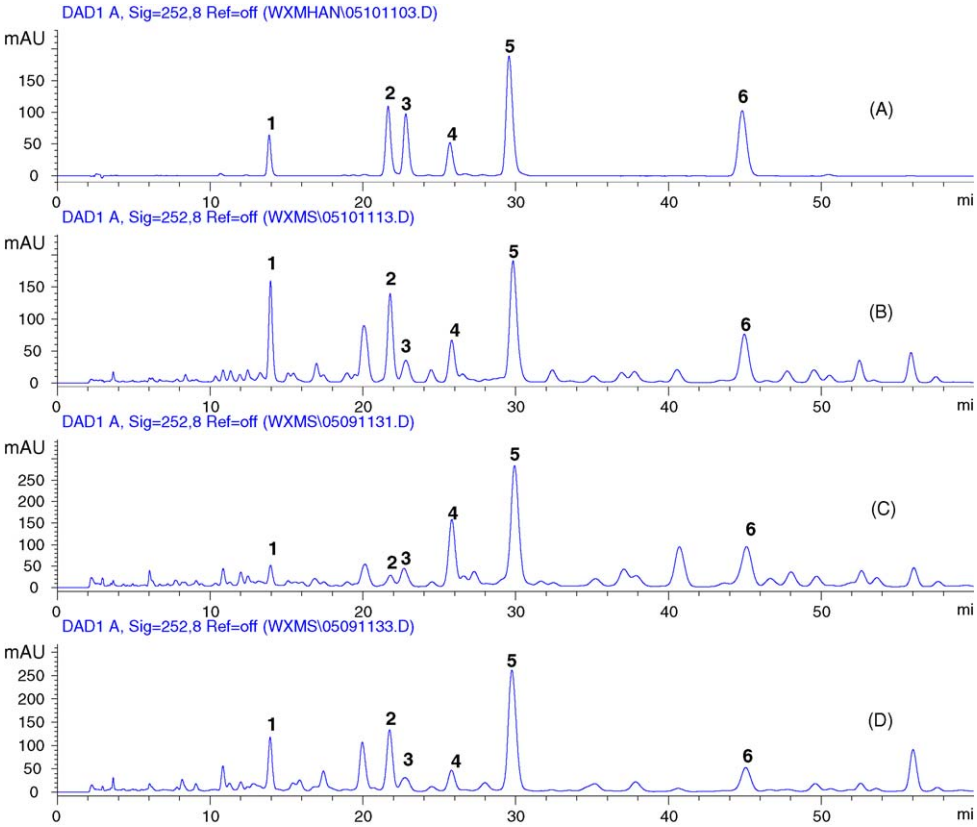

Fig. 2. HPLC chromatograms of standard mixture (A), sample no. 4 (B), sample no. 30 (C), sample no. 36 (D). (**1**) ganoderic acid C2; (**2**) ganoderic acid B; (**3**) ganoderic acid AM1; (**4**) ganoderic acid K; (**5**) ganoderic acid H; (**6**) ganoderic acid D.

Table 2
Linear relation between peak area and concentration ($n = 7$)

Compound	Regression equation	r^2	F-test[a]		F-test[b]		Linear range (μg/ml)	LOD (μg/ml)	LOQ (μg/ml)
			F	p-value	F	p-value			
1	$y = 6.98x - 8.05$	0.999	68175.1	<0.0001	0.14	0.9804	17–510	0.23	0.68
2	$y = 7.37x - 14.44$	0.999	62554.9	<0.0001	0.61	0.6925	18.5–555	0.25	0.74
3	$y = 5.12x - 8.72$	0.999	232327	<0.0001	1.49	0.2538	18–540	0.24	0.72
4	$y = 6.50x - 13.81$	0.999	82261.8	<0.0001	0.29	0.9108	17.5–525	0.23	0.70
5	$y = 5.05x - 30.05$	0.999	100460	<0.0001	0.75	0.6015	67–2010	0.22	0.67
6	$y = 7.19x - 13.36$	0.999	88512.6	<0.0001	0.42	0.8254	22.5–675	0.30	0.90

In the regression equation $y = ax + b$, x refers to the concentration of the triterpenoid (μg/ml), y the peak area, and r^2 is the correlation coefficient of the equation. LOD, limit of detection; LOQ, limit of quantification.
[a] F-test for r^2 of regression equation.
[b] F-test for lack of fit.

Table 3
Intra- and inter-day repeatability for six triterpenoids in *G. lucidum*

Compound	Intra-day ($n = 6$)						Inter-day ($n = 3$)	
	Day 1		Day 2		Day 3		Mean ± S.D.[a]	R.S.D. (%)
	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.[a]	R.S.D. (%)		
1	585.4 ± 8.9	1.52	591.4 ± 5.9	0.99	581.1 ± 4.6	0.80	585.9 ± 5.2	0.89
2	747.3 ± 11.0	1.47	753.4 ± 10.4	1.39	745.8 ± 7.5	1.01	748.8 ± 4.1	0.54
3	412.8 ± 5.1	1.22	416.4 ± 2.4	0.59	413.2 ± 5.2	1.26	414.2 ± 2.0	0.48
4	571.7 ± 7.0	1.22	571.7 ± 2.5	0.44	568.3 ± 2.4	0.42	570.3 ± 2.5	0.43
5	2225.9 ± 19.6	0.88	2247.7 ± 43.5	1.94	2251.7 ± 44.9	1.99	2241.8 ± 13.8	0.47
6	727.4 ± 9.5	1.31	733.8 ± 5.9	0.82	722.2 ± 6.2	0.86	727.8 ± 5.9	0.81
Total	5270.6 ± 53.0	1.00	5312.6 ± 57.4	1.08	5281.2 ± 65.1	1.23	5288.7 ± 22.9	0.43

[a] Data were microgram triterpenoids per gram crude drug.

Table 4
Recovery of the six triterpenoids in *G. lucidum* ($n = 3$)

Compound	Added (μg/ml)	Detected (μg/ml)[a]	Recovery (%)[b]	R.S.D. (%)
	165.2	165.2	100.00	1.87
1	66.1	63.9	96.77	4.31
	33.0	30.5	96.26	3.91
	169.6	172.0	101.40	0.69
2	67.8	68.4	100.75	1.40
	33.9	32.4	95.65	3.93
	171.6	177.4	103.37	1.08
3	68.6	67.7	98.69	3.18
	34.3	32.0	93.14	2.17
	172.4	172.8	100.22	1.39
4	69.0	71.0	102.95	2.77
	34.5	32.6	97.43	4.20
	642.8	642.9	100.02	1.55
5	257.1	253.0	98.41	1.26
	128.6	127.2	98.90	1.88
	211.6	215.7	101.92	0.86
6	84.6	82.9	97.98	4.25
	42.3	40.2	95.04	3.52

[a] Calculated by subtracting the total amount after spiking from the amount in the herb before spiking. Data were means of three experiments.
[b] Calculated as detected amount/added amount × 100%. Data were means of three experiments.

triterpenoids differed significantly in different species because of the difference in genetic source, cultivating conditions, or manufacturing process. These results showed that samples of *G. lucidum* and *Ganoderma tropicum* contained all six triterpenoids, but *Ganoderma sinense*, *Ganoderma amboinense and Ganoderma sessile* only contain part of these triterpenoids, and *G. lucidum* had the highest content of total triterpenoids. Ganoderic acid H is the major compound in all samples. The average content of total triterpenoids in *G. lucidum* (sample nos. 2–10) that were cultivated under good agricultural practice (GAP) by Green Valley Pharmaceutical Co. is 1.4 times higher than other samples (samples no. 11–22), which indicated the former might have better quality than the latters.

Table 5
Stability of mixture stock solution and sample solution stored at 20–25 °C within 3 days

Compound	Mixture stock solution		Sample solution	
	Mean ± S.D.[a]	R.S.D. (%)	Mean ± S.D.[b]	R.S.D. (%)
1	510.0 ± 8.0	1.58	584.8 ± 9.4	1.60
2	553.8 ± 6.0	1.09	748.4 ± 12.2	1.64
3	539.7 ± 3.9	0.73	413.8 ± 5.8	1.41
4	525.9 ± 3.1	0.73	568.3 ± 4.4	0.77
5	2007.4 ± 22.7	1.13	2245.7 ± 57.9	2.58
6	674.3 ± 9.1	1.34	725.6 ± 11.4	1.56

[a] Data were microgram triterpenoids per milliliter.
[b] Data were microgram triterpenoids per gram crude drug.

Table 6
Contents of triterpenoids in different *Ganoderma* samples ($n = 3$)

Sample no.	Species	Origin	Content (μg/g)[a]						
			(1)	(2)	(3)	(4)	(5)	(6)	Total
1	*G. lucidum*	Control sample[b]	199.0 ± 4.7	239.5 ± 2.6	207.3 ± 4.2	178.1 ± 3.4	1625.7 ± 31.3	532.4 ± 14.2	3036.0 ± 38.9
2[c]	*G. lucidum*	Wuyi mountain, Fujian province	385.7 ± 3.0	592.9 ± 8.8	619.2 ± 8.0	460.5 ± 11.2	3860.2 ± 62.2	951.7 ± 3.5	6870.3 ± 90.2
3[c]	*G. lucidum*	Wuyi mountain, Fujian province	1423.6 ± 21.6	1759.7 ± 26.8	818.8 ± 5.7	1091.7 ± 41.8	5224.3 ± 158.8	1233.6 ± 6.6	11551.9 ± 238.6
4[c]	*G. lucidum*	Wuyi mountain, Fujian province	217.2 ± 3.9	356.2 ± 1.0	829.1 ± 7.4	774.3 ± 11.5	3601.2 ± 28.4	748.4 ± 5.2	6526.4 ± 32.6
5[c]	*G. lucidum*	Wuyi mountain, Fujian province	164.5 ± 2.4	312.0 ± 1.0	568.2 ± 7.2	646.9 ± 11.9	2304.6 ± 29.0	505.2 ± 10.4	4501.4 ± 43.3
6[c]	*G. lucidum*	Wuyi mountain, Fujian province	173.6 ± 2.3	397.1 ± 7.9	725.3 ± 13.2	913.2 ± 10.2	1509.8 ± 8.1	773.2 ± 7.5	4492.2 ± 21.0
7[c]	*G. lucidum*	Wuyi mountain, Fujian province	76.0 ± 1.5	198.9 ± 3.9	799.9 ± 6.6	624.2 ± 10.6	596.8 ± 16.3	507.3 ± 7.4	2803.1 ± 17.7
8[c]	*G. lucidum*	Wuyi mountain, Fujian province	399.0 ± 1.3	574.8 ± 5.6	413.2 ± 3.9	470.8 ± 17.7	3096.1 ± 28.1	519.1 ± 2.6	5473.0 ± 33.9
9[c]	*G. lucidum*	Wuyi mountain, Fujian province	284.7 ± 4.2	424.9 ± 2.1	553.0 ± 4.0	835.2 ± 77.1	3076.2 ± 30.8	512.5 ± 13.8	5686.7 ± 82.2
10[c]	*G. lucidum*	Wuyi mountain, Fujian province	579.2 ± 8.7	739.5 ± 10.8	410.1 ± 6.1	571.3 ± 10.8	2213.4 ± 20.3	722.2 ± 11.6	5235.8 ± 54.2
11[c]	*G. lucidum*	Shouning Conuty, Fujian province	445.1 ± 9.2	864.5 ± 19.5	478.2 ± 2.4	526.5 ± 4.3	4373.9 ± 91.5	1452.5 ± 7.6	8140.9 ± 104.7
12[c]	*G. lucidum*	Huangshan mountain, Anhui province	755.8 ± 11.2	1300.5 ± 18.4	719.5 ± 12.9	913.6 ± 16.5	3762.1 ± 33.2	729.9 ± 20.3	8181.4 ± 60.9
13	*G. lucidum*	Urumqi, Xinjiang province	160.2 ± 4.5	339.3 ± 3.0	567.0 ± 3.9	783.6 ± 28.1	2721.9 ± 61.9	657.1 ± 3.2	5229.2 ± 79.6
14	*G. lucidum*	Nanjing city, Jiansu province	407.8 ± 7.7	627.6 ± 14.4	354.5 ± 3.1	630.6 ± 3.6	1951.6 ± 24.4	401.5 ± 13.7	4373.7 ± 42.9
15	*G. lucidum*	Kunming city, Yunnan province	299.4 ± 5.7	499.6 ± 5.3	677.5 ± 9.0	925.9 ± 2.0	3690.9 ± 110.1	816.3 ± 7.9	6909.6 ± 130.0
16	*G. lucidum*	Qinhuangdao city, Hebei province	195.8 ± 6.1	384.1 ± 2.7	212.1 ± 4.6	412.0 ± 5.7	1529.9 ± 21.7	518.7 ± 10.2	3252.8 ± 30.6
17	*G. lucidum*	Jinzhou city, Liaoning province	150.0 ± 7.2	384.3 ± 8.4	202.6 ± 2.7	389.0 ± 4.7	1556.3 ± 8.1	656.3 ± 17.3	3338.5 ± 16.1
18	*G. lucidum*	Lhasa city, Tibet province	113.8 ± 2.9	255.6 ± 2.3	250.4 ± 4.4	298.1 ± 3.4	915.9 ± 10.9	275.0 ± 2.4	2108.9 ± 22.4
19	*G. lucidum*	Changchun city, Jilin province	133.0 ± 2.6	183.6 ± 3.1	385.8 ± 11.2	1194.1 ± 17.2	2332.7 ± 21.4	535.7 ± 5.8	4764.8 ± 29.9
20	*G. lucidum*	Beijing	383.5 ± 5.8	623.5 ± 9.8	413.3 ± 3.2	800.5 ± 14.3	2445.8 ± 42.4	529.4 ± 4.3	5195.9 ± 49.0
21	*G. lucidum*	Hechi city, Guangxi province	643.7 ± 6.7	1018.5 ± 8.1	806.3 ± 1.1	742.8 ± 6.1	2998.1 ± 21.1	601.3 ± 3.1	6810.8 ± 46.2
22	*G. lucidum*	Wuhan city, Hubei province	139.1 ± 1.2	197.1 ± 1.9	209.1 ± 1.0	214.5 ± 1.6	876.4 ± 3.0	222.4 ± 4.3	1858.5 ± 11.2
23	*G. sinense*	Guiyang city, Guizhou province	38.8 ± 1.3[d]	30.0 ± 0.4[d]	96.0 ± 1.2	–	359.4 ± 4.6	–	520.2 ± 7.3
24	*G. sinense*	Yining city, Xinjiang province	37.8 ± 1.0[d]	–	–	–	35.3 ± 0.7[d]	–	73.1 ± 0.3
25	*G. sinense*	Guiyang city, Guizhou province	90.4 ± 1.7	–	–	30.3 ± 1.4[d]	31.8 ± 0.7[d]	–	152.5 ± 1.7
26	*G. sinense*	Guangzhou city, Guangdong province	21.0 ± 0.7[d]	19.5 ± 0.9[d]	–	19.3 ± 1.4[d]	57.8 ± 2.1[d]	–	117.7 ± 2.1
27[c]	*G. sinense*	Fuzhou city, Fujian province	46.1 ± 1.6	73.4 ± 1.9	55.2 ± 1.7	78.9 ± 0.6	333.7 ± 5.4	100.6 ± 3.6	687.8 ± 5.7
28	*G. sinense*	Hechi city, Guangxi province	684.8 ± 17.0	191.8 ± 2.6	59.6 ± 1.9	765.8 ± 12.9	216.1 ± 6.3	–	1918.1 ± 35.2
29	*G. sinense*	Yandang mountain, Zhejiang province	37.8 ± 1.5[d]	–	–	–	–	–	37.8 ± 1.5
30[c]	*G. amboinense*	Fuzhou city, Fujian province	290.0 ± 6.0	303.4 ± 2.2	733.6 ± 11.5	1913.3 ± 14.9	5734.8 ± 98.6	1538.6 ± 24.6	10513.8 ± 97.4
31	*G. amboinense*	Lhasa city, Tibet province	–	–	327.2 ± 1.2	138.4 ± 5.5	271.8 ± 6.1	–	737.4 ± 0.9
32	*G. amboinense*	Fuhai County, Xinjiang province	281.6 ± 0.6	350.4 ± 5.4	509.4 ± 6.3	601.6 ± 18.3	3597.8 ± 24.7	1185.4 ± 4.1	6526.3 ± 15.8
33	*G. sessile*	Chengdu city, Sichuan province	106.0 ± 3.4	197.6 ± 2.6	157.6 ± 3.8	140.0 ± 3.9	583.1 ± 11.2	195.8 ± 1.0	1380.3 ± 25.0
34	*G. sessile*	Lhasa city, Tibet province	–	–	–	70.8 ± 0.9	96.5 ± 1.2[d]	–	167.3 ± 0.5
35[c]	*G. atrum*	Fuzhou city, Fujian province	33.9 ± 1.8[d]	35.1 ± 1.0[d]	–	35.0 ± 1.0[d]	295.4 ± 8.1	108.4 ± 1.0	507.8 ± 8.2
36[c]	*G. tropicum*	Fuzhou city, Fujian province	782.6 ± 15.1	1271.1 ± 19.1	584.6 ± 3.2	605.0 ± 15.4	4916.3 ± 48.2	814.5 ± 20.2	8974.1 ± 78.9

(–) Lower than test limit and could not be quantified.

[a] Data were expressed as mean ± S.D. of three experiments.

[b] No. 1 sample is a control sample according to China Pharmacopoeia, purchased from National Institute for the Control of Pharmaceutical and Biological Products.

[c] The herb samples of *Ganoderma* were kindly provided by Shanghai Green Valley Holding CO., Ltd. and samples 2–10 was cultured in compliance with good agricultural practice. The other samples were purchased from various drug stores in China.

[d] Out of linear range.

Both *G. lucidum* and *G. sinense* are the official two species of Lingzhi recorded in Chinese Pharmacopoeia (2005 edition) and they were considered to have the same therapeutic effects [19]. But the average content of total triterpenoids in *G. lucidum* was 10 times higher than that in *G. sinense*, which indicated the therapeutic effects of these two species might be quite different. Therefore, they are recommended to use as two different herbs in clinics.

4. Conclusion

This is the first report on the simultaneous determination of six major triterpenoids in *G. lucidum*, which proved to be simple, rapid, accurate and reliable. Under the multiple optimized HPLC conditions, six triterpenoids were totally separated and eluted individually within 50 min. The validation procedure confirmed that this method was not only suitable for the analysis of these triterpenoids but also for the quality evaluation of *Ganoderma* products.

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