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Development and validation of an HPLC-DAD method for simultaneous quantification six ginsenosides in cultivated *Panax vietnamensis var. fuscidiscus*

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ABSTRACT

Background: Panax vietnamensis var. fuscidiscus K. Komatsu, S. Zhu & S.Q. Cai is a rare medicinal plant endemic to Vietnam, known for its rich profile of pharmacologically active ginsenosides. Objective: This study aimed to develop and validate a high-performance liquid chromatography method with diode array detection (HPLC-DAD) for the simultaneous quantification of six key ginsenosides: Ginsenoside Rg1, Majonoside R2, Ginsenoside Rb1, Notoginsenoside R4, Notoginsenoside Fc, and Ginsenoside Rd. Methods: Chromatographic separation was achieved using a Shim-pack GIST C18 column (250 × 4.6 mm, 5 µm) on a Shimadzu LC-2030C 3D Plus system. A gradient elution with water and acetonitrile was employed, and detection was performed at 195 nm. All six compounds were baseline-separated within 50 minutes ($R_s > 1.5$). The extraction process was optimized using ultrasound-assisted extraction with ethanol:water (1:1), a material-to-solvent ratio of 1/50, 20-minute extraction time, repeated twice. Results: The method demonstrated good specificity, linearity ($R^2 > 0.999$), and precision (RSD < 2.7%). Limits of detection ranged from 0.012% to 0.021%, and limits of quantification from 0.040% to 0.068%. Accuracy, confirmed by recovery tests, ranged from 97.08% to 102.89%. Conclusion: The developed HPLC-DAD method is accurate, precise, and reproducible, providing a reliable analytical tool for the quality assessment and standardization of Panax vietnamensis var. fuscidiscus K. Komatsu, S. Zhu & S.Q. Cai and its derived herbal products.

Keywords: *Ginsenosides, Panax, Panax Vietnamensis, Panax vietnamensis var. fuscidiscus, HPLC-DAD*

1. INTRODUCTION

Panax vietnamensis var. fuscidiscus K. Komatsu, S. Zhu & S.Q. Cai (SLC) is a subspecies of Vietnamese ginseng that was first discovered in Lai Châu Province in 2013 [1]. This species has attracted increasing scientific and commercial interest due to its abundant bioactive constituents, particularly dammarane-type saponins such as ginsenosides and majonosides, which exhibit immunomodulatory, antioxidant, hepatoprotective, neuroprotective, and anti-inflammatory activities. Among them, six characteristic compounds - Ginsenoside Rg1 (G-Rg1), Majonoside R2 (M-R2), Ginsenoside Rb1 (G-Rb1), Notoginsenoside R4 (N-R4), Notoginsenoside Fc (N-Fc), and Ginsenoside Rd (G-Rd) - are considered key chemical markers for quality assessment of *Vietnamese ginseng* species [2 - 5].

However, due to the growing market demand, wild SLC populations are being overexploited, leading to a serious risk of depletion. In this context, the conservation, cultivation, expansion, and sustainable development of SLC have become urgent priorities, contributing to the preservation of this rare genetic resource and the promotion of local economic growth. Although several analytical methods for ginsenoside quantification in Panax species have been reported, including HPLC and LC-MS/MS [6 - 8], no standardized or validated protocol currently exists for the simultaneous determination of the characteristic ginsenosides in SLC. Therefore, establishing a simultaneous quantification method for six characteristic ginsenosides in SLC using high-performance liquid

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chromatography coupled with a diode array detector (HPLC-DAD) is essential, as it supports the quality control and authentication of cultivated SLC products available on the market.

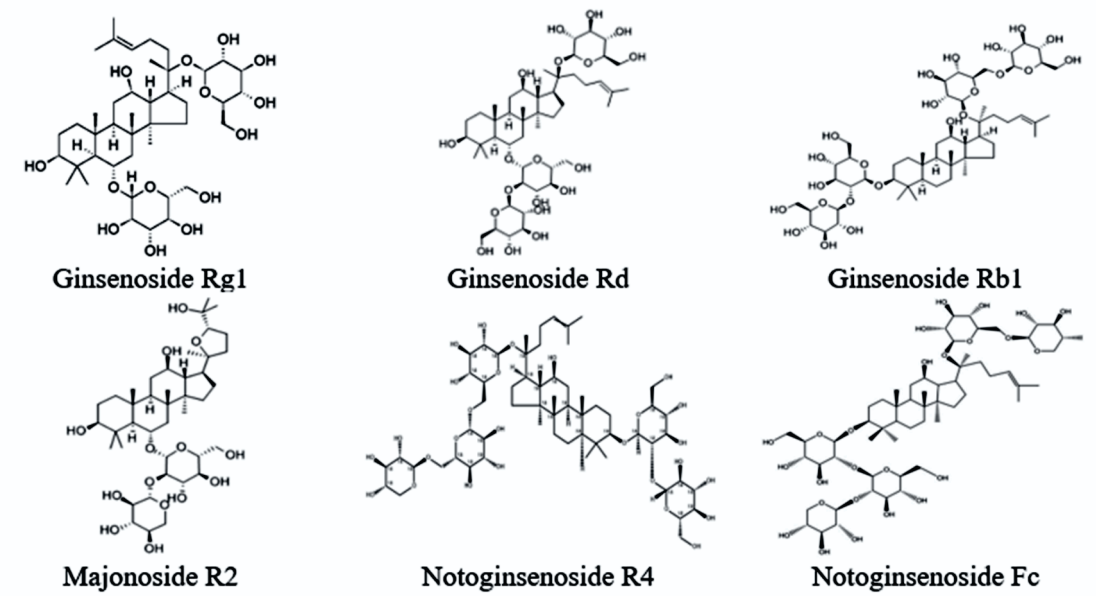

Figure 1. Chemical structures of six ginsenosides used for quantitative analysis in SLC (G-Rg1, M-R2, G-Rb1, N-R4, N-Fc, and G-Rd)

2. MATERIALS AND METHODOLOGY

2.1. Materials

The underground parts of five-year-old cultivated SLC were provided by Thai Minh Company, collected from Ban Sang Phin, Sa De Phin Commune, Sin Ho District, Lai Chau Province, Vietnam. The plant material was authenticated by DNA identification at the University of Science, Ho Chi Minh City. After preliminary processing to achieve a moisture content below 12%, the samples were pulverized and sieved through a 500 µm mesh, then stored in sealed bags under dry and well-ventilated conditions, protected from direct sunlight and humidity.

2.2. Chemicals and Equipment

2.2.1. Chemicals

Reference standards of ginsenosides were obtained as follows: ginsenoside Rg1 (Sigma-Aldrich, Lot No. 0000374177, purity 100.0%), majonoside R2 (Ho Chi Minh City Institute of Drug Quality Control, Lot No. R2-2022, purity 96.5%), ginsenoside Rb1 (HWI Group, Lot No. HWI03437, purity 99.7%), ginsenoside Rd (Sigma-Aldrich, Lot No. BCCL9322, purity 99.7%), notoginsenoside R4 (CATO Research Chemicals Inc., Lot No. 0711-SA-7171, purity 98.845%), and notoginsenoside Fc (CATO Research Chemicals Inc., Lot No. 0129-RD-0025, purity 98.9%).

Ethanol (Merck, ≥ 99.9%), methanol (Merck, ≥ 99.8%), acetonitrile (Merck, ≥ 99.0%), chloroform (Merck, ≥ 99.8%), HPLC-grade methanol (Fisher, ≥ 99.0%), and HPLC-grade acetonitrile (Fisher, ≥ 99.0%) were used in this study. Double-distilled water was used throughout the experiments.

2.2.2. Equipments

Analyses were performed on a Shimadzu LC-2030C 3D Plus HPLC system equipped with a Shim-pack GIST C18 column (250 × 4.6 mm; 5 µm) and a Shim-pack GIST C18 guard column (10 × 4.0 mm; 5 µm). Additional equipment included a Sonorex RK-1028H ultrasonic bath, a Memmert WB-14 water bath, analytical balances (4- and 5-decimal place), a Gallenkamp vacuum oven, micropipettes (10 - 100 µL, 20 - 200 µL, 100 - 1,000 µL), and standard glassware (beakers, pipettes, graduated cylinders, etc.).

2.3. Methodology

2.3.1. Optimization of the HPLC mobile phase program for simultaneous quantification of six Ginsenosides in SLC

The simultaneous quantification of six ginsenosides in SLC was performed using an HPLC-DAD system (Shimadzu LC-2030C 3D Plus) with a Shim-pack GIST C18 column (250 × 4.6 mm; 5 µm) and guard

column (10 × 4.0 mm; 5 µm). The column temperature was maintained at 50°C, the detection wavelength was set at 195 nm, and the injection volume was 40 µL. Several mobile phase programs were tested to ensure complete separation of the six ginsenoside peaks from other components, with chromatographic parameters meeting system suitability requirements.

2.3.2. Optimization of the extraction procedure

Solvent selection

Approximately 100 mg of SLC powder was accurately weighed into a 15 mL centrifuge tube, followed by the addition of 5 mL of methanol. Ultrasonic extraction was conducted for 20 min, the extract was centrifuged and cooled, and the supernatant was transferred to a 5 mL volumetric flask and made up to volume. The solution was filtered through a 0.45 µm membrane filter and analyzed under the optimized HPLC conditions. The same procedure was repeated using ethanol, acetonitrile, water, and mixed solvent systems (MeOH:H_2O and EtOH:H_2O, ACN:H_2O) at ratios of 1:1, 3:7, and 7:3 (v/v). The six ginsenoside contents obtained from different solvents were compared to determine the optimal extraction solvent.

Sample-to-solvent ratio selection

Using the optimal solvent from the previous step, different sample-to-solvent ratios were investigated (five ratios in total). Each sample was ultrasonically extracted for 20 min, centrifuged, and the extract volume was adjusted to 5 mL, filtered through a 0.45 µm membrane, and analyzed by HPLC. The ginsenoside contents at various ratios were compared to select the most suitable sample-to-solvent ratio.

Extraction time optimizing

Approximately 100 mg of SLC powder was extracted with 5 mL of the optimal solvent at different ultrasonic extraction times: 2, 5, 10, 20, 30, 40, and 60 min. Extracts were centrifuged, cooled, adjusted to 5 mL, filtered (0.45 µm), and analyzed under the optimized HPLC conditions. The extraction time yielding the highest ginsenoside content was selected as optimal.

Extract- repetitions selecting

The extraction process was evaluated for one, two, and three successive extractions. For each cycle, 100 mg of SLC powder was extracted with 5 mL of the selected solvent under ultrasonic conditions for 20 min. Each extract was centrifuged, cooled, adjusted to 5 mL, filtered (0.45 µm), and analyzed by HPLC. The ginsenoside contents from each extraction cycle were compared to determine the optimal number of extractions.

2.3.3. Validation of the simultaneous quantification method for six ginsenosides in SLC by HPLC-DAD

The simultaneous quantification method for six ginsenosides in SLC using HPLC-DAD was validated in accordance with ICH guidelines, evaluating system suitability, specificity, linearity, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ) [9].

3. RESULTS

3.1. Mobile phage program investigation

The gradient elution programs were investigated using solvent A (H_2O) and solvent B (acetonitrile, ACN). The optimal mobile phase program was selected as shown in Table 1. Under these conditions, six ginsenosides - Ginsenoside Rg1, Majonoside R2, Ginsenoside Rb1, Notoginsenoside R4, Notoginsenoside Fc, and Ginsenoside Rd - were completely separated, with the resolution (Rs) between the adjacent peaks of Notoginsenoside R4 and Notoginsenoside Fc meeting the requirement of Rs > 1.5.

Table 1. Mobile phage program

Time (min)	Channel A (H_2O)%	Channel (ACN)%	Flow rate (mL.min^{-1})
0.01	90	10	1
5.00	90	10	1
35.00	40	60	1
36.00	5	95	1
45.00	5	95	1
46.00	90	10	1
50.00	90	10	1

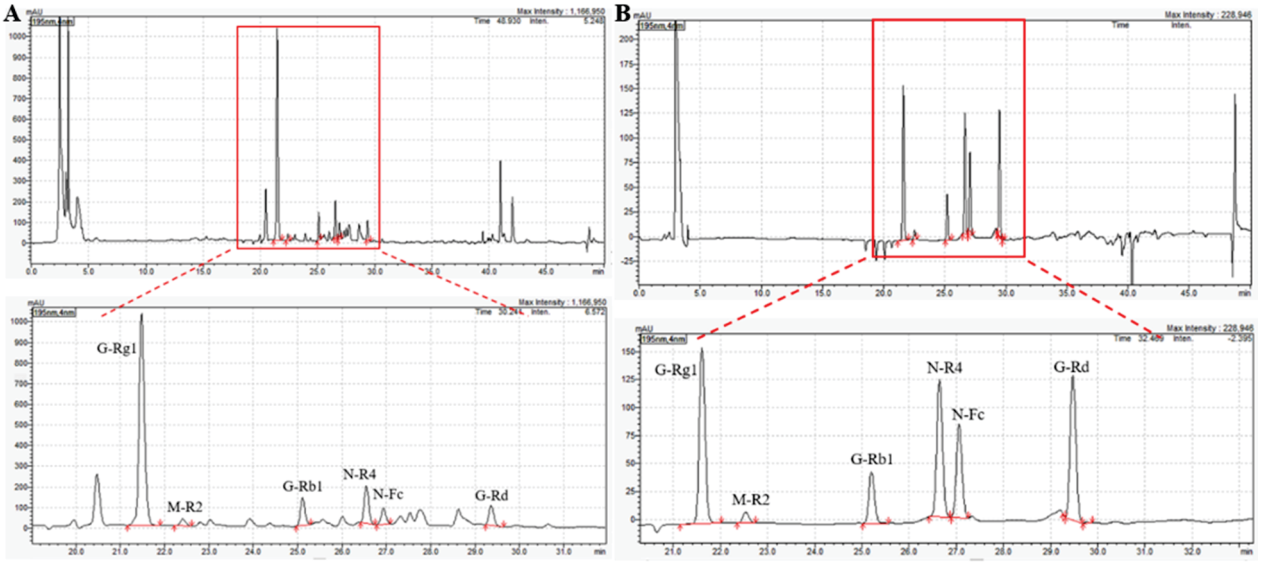

Figure 2. HPLC chromatogram of SLC sample obtained using the optimized gradient mobile-phase program (A), and HPLC chromatogram of the mixed standard solution containing six ginsenosides (G-Rg1, M-R2, G-Rb1, N-R4, N-Fc, and G-Rd) (B)

3.2. Extracting procedure optimization

Solvent selection

A series of solvents with increasing polarity was investigated. The contents of six ginsenosides in SLC were quantified using the HPLC-DAD method. The results obtained from extraction with 13 different solvent systems are presented in Figure 3.

The results showed that solvent mixtures of ethanol:water (EtOH:H_2O) and methanol:water (MeOH:H_2O) exhibited strong extraction efficiency due to their intermediate polarity, which is suitable for dissolving amphiphilic compounds such as saponins [10]. The EtOH:H_2O mixture at a 1:1 ratio was selected because it provided consistently high extraction yields for all six compounds, with the highest contents observed for M-R2 (~4.8%) and G-Rg1 (~2.8%), while the remaining saponins ranged between 2 - 4%. Moreover, EtOH:H_2O is a safe and low-toxicity solvent, compliant with the Vietnamese Pharmacopoeia V regulations for herbal extraction.

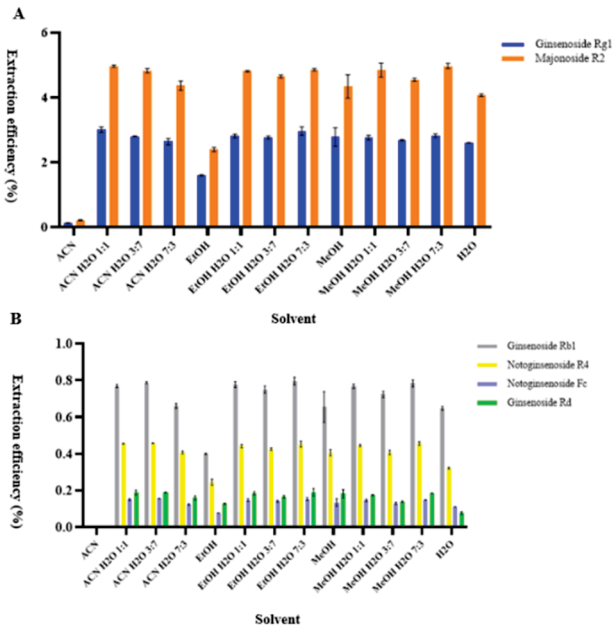

Figure 3. Extraction efficiencies of G-Rg1 and M-R2 from SLC using different solvent systems (A) and extraction efficiencies of G-Rb1, N-R4, N-Fc, and G-Rd from SLC using various solvent systems (B)

Investigation of the ratio between sample mass and solvent volume

The extraction efficiency was further examined at five different ratios of sample mass to solvent volume (1/10, 1/20, 1/50, 1/100, and 1/200, w/v) using the EtOH:H_2O (1:1) solvent system. The results are presented in Figure 4.

The results shown in Figure 4 indicate the contents of six ginsenosides determined at five different solvent-to-sample ratios. Among them, the 1/50 ratio yielded the highest total ginsenoside content. Therefore, the solvent-to-sample ratio of 1/50 (w/v) was selected as the optimal condition for extracting SLC samples.

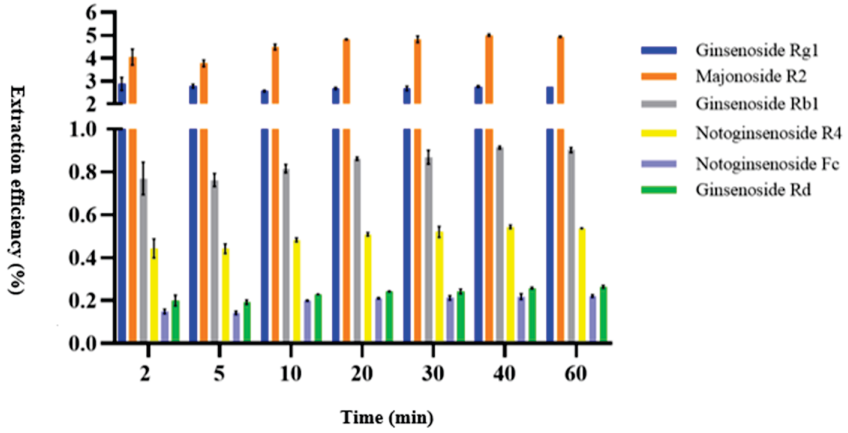

Figure 4. Effect of the sample-to-solvent ratio on the extraction efficiency of six ginsenosides from SLC. Ethanol:water (1:1, v/v) was used as the extraction solvent

Investigation of the extraction time

The extraction time was further optimized by quantifying six ginsenosides in SLC using the HPLC-DAD method after ultrasonic extraction for 2, 5, 10, 20, 30, 40, and 60 minutes. The results are presented in Figure 5.

The results of the extraction time study showed that the contents of the six ginsenosides increased progressively from 2 to 20 minutes, after which they stabilized and showed no significant further increase. The highest total ginsenoside content was observed at 20 minutes, particularly for M-R2. Therefore, an extraction time of 20 minutes was selected as the optimal condition for preparing SLC samples.

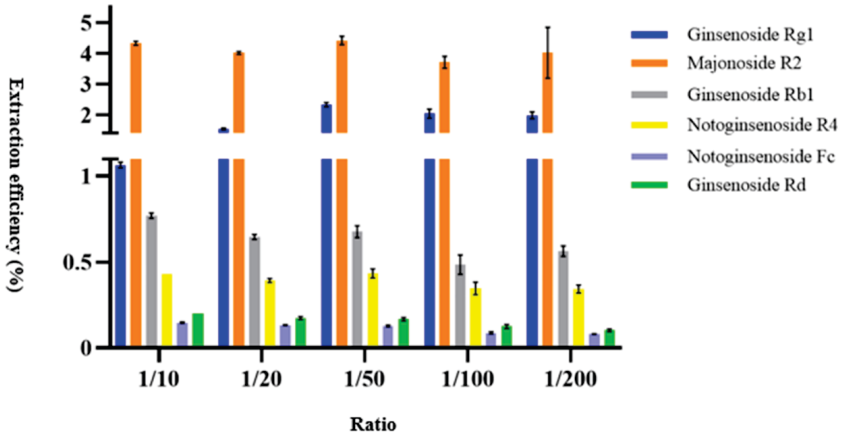

Figure 5. Effect of extraction time on the yields of six ginsenosides in SLC using ultrasonic-assisted extraction with ethanol:water (1:1, v/v)

The effect of the number of extraction cycles on ginsenoside yield was evaluated by quantifying six ginsenosides in SLC using the HPLC-DAD method after one, two, and three extraction cycles. The results are summarized in Figure 6.

The results shown in Figure 6 indicate that the six ginsenosides were essentially exhausted by the second extraction. The first extraction recovered the majority of the analytes. During the second extraction, although the ginsenoside contents

decreased significantly compared to the first cycle, several marker compounds, including G-Rg1, M-R2, and G-Rb1, were still detectable. In the third extraction, the contents of marker compounds M-R2, G-Rb1, N-R4, N-Fc, and G-Rd fell below the quantification limit. Therefore, two extraction cycles were selected as the optimal procedure for preparing SLC samples for analysis.

In this study, the extraction conditions for SLC (*Panax vietnamensis var. fuscidiscus*) were optimized to maximize the yield of six characteristic ginsenosides. When compared to extraction protocols reported for other Panax species, such as *P. ginseng* and *P. notoginseng* [6 - 8], several similarities and differences were observed.

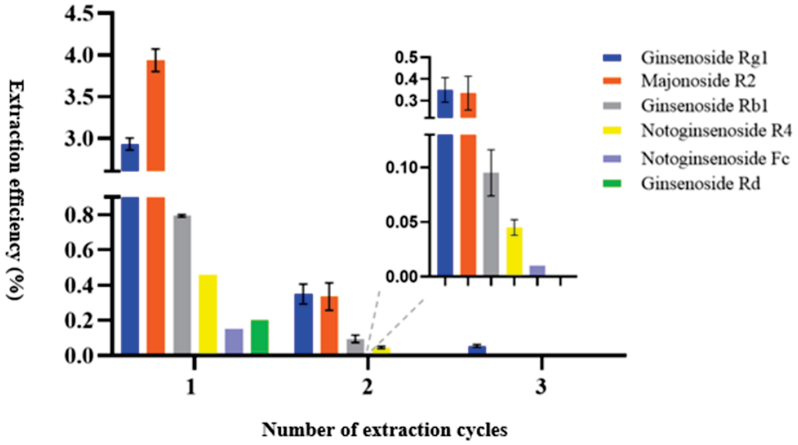

Figure 6. Effect of the number of extraction cycles on the yields of six ginsenosides in SLC using ethanol:water (1:1, v/v) and ultrasonic-assisted extraction

3.3. Validation of the simultaneous quantification method for six ginsenosides in SLC by HPLC-DAD

3.3.1. System suitability

System suitability of the simultaneous quantification method for six ginsenosides was evaluated, and the results are presented in Table 2. The relative standard deviations (RSD) of retention times (tR) and peak areas (S) for all six ginsenosides were less than 2%. The resolution (Rs) values were all greater than 1.5, the tailing factors ranged from 1.0 to 1.5, and the theoretical plate numbers exceeded 120,000. These results demonstrate that the HPLC-DAD method for the simultaneous quantification of six ginsenosides in SLC meets system suitability criteria according to ICH guidelines.

Table 2. System suitability results for the simultaneous quantification of six ginsenosides in SLC using HPLC-DAD

Compounds	n = 7	t_R (min)	S (mAU.s)	R_s (USP)	Tailing F.	NTP (USP)
G-Rg1	Average	21.574 ± 0.008	1,323,205 ± 886.30	--	1.125	126,914
	RSD	0.035	0.067			
M-R2	Average	22.505 ± 0.007	87.963 ± 147.91	3.718	1.093	120,907
	RSD	0.031	0.168			
G-Rb1	Average	25.167 ± 0.009	372.767 ± 402.95	10.843	1.110	188,288
	RSD	0.034	0.108			
N-R4	Average	26.611 ± 0.009	1.031.993 ± 3656.80	6.120	1.096	196,594
	RSD	0.033	0.354			
N-Fc	Average	27.028 ± 0.009	688.416 ± 1.655.65	1.745	1.051	206,378
	RSD	0.034	0.241			
G-Rd	Average	29.443 ± 0.009	1.187.861 ± 622.98	11.570	1.097	214,899
	RSD	0.031	0.052			

3.3.2. Specificity

The specificity of the method was evaluated by analyzing a blank sample, a standard solution, a test sample, and a spiked test sample. The resulting

chromatograms are shown in Figure 7. No peaks in the blank sample coincided with the retention times of the six ginsenosides - G-Rg1, M-R2, G-Rb1, N-R4, N-Fc, and G-Rd - observed in the standard and test samples. The test sample chromatogram showed peaks at retention times corresponding to those of the analytes in the standard solution. In the spiked test sample, both peak heights and areas increased compared to the test sample, confirming proper identification. The retention times of the six ginsenosides were as follows: G-Rg1 (21.6 min), M-R2 (22.5 min), G-Rb1 (25.2 min), N-R4 (26.7 min), N-Fc (27.1 min), and G-Rd (29.4 min). All peaks were baseline-separated in both standard and test samples, and peak purity assessments met the required criteria. These results demonstrate that the method is specific for the simultaneous quantification of six ginsenosides in SLC.

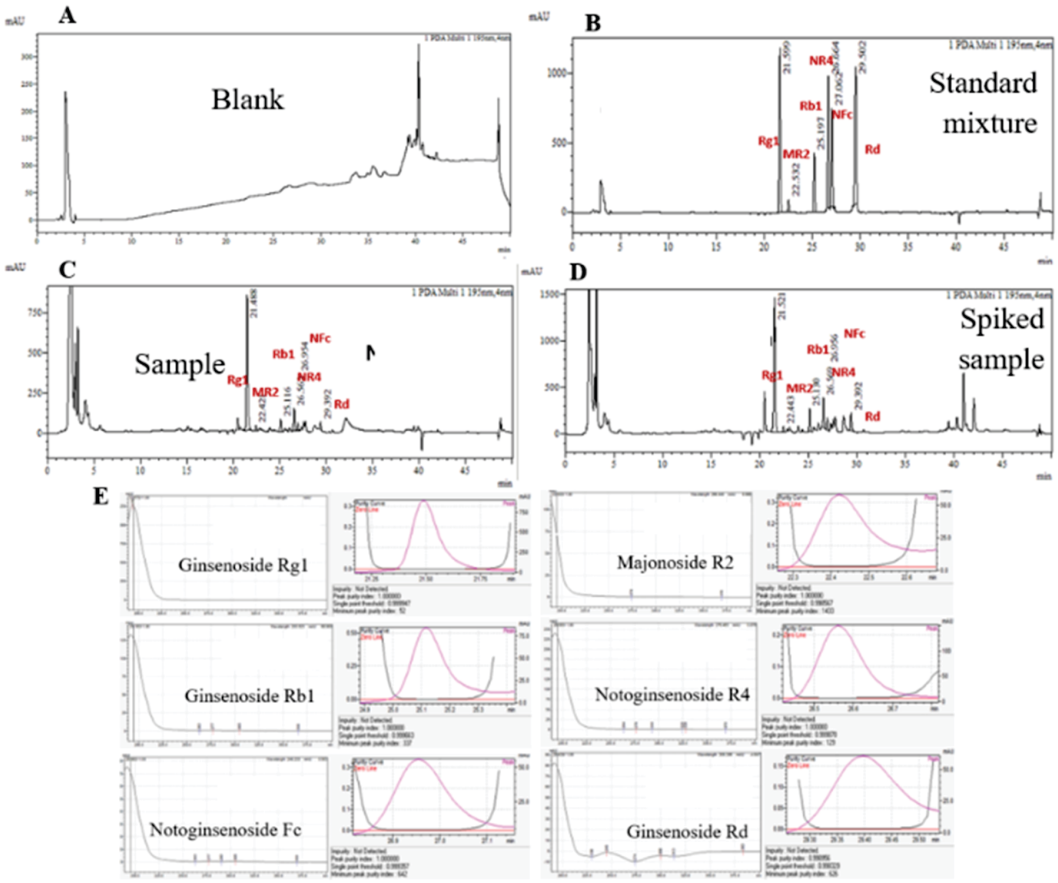

Figure 7. HPLC-DAD chromatograms of blank (A), standard mixture (B), SLC sample extract (C), and spiked SLC sample extract (D) for the simultaneous quantification of six ginsenosides (G-Rg1, M-R2, G-Rb1, N-R4, N-Fc, and G-Rd) from SLC. (E) UV spectra and peak purity analysis of the six in SLC sample using HPLC-DAD

3.3.3. Linearity

The linearity of the developed HPLC-DAD method for the simultaneous quantification of six ginsenosides in SLC was evaluated, and the results are summarized in Table 3.

The calibration curves for all six ginsenosides exhibited excellent linearity with correlation coefficients (R^2) greater than 0.998, demonstrating the method's suitability for accurate quantification of ginsenosides in SLC samples.

Table 3. Calibration equations, linear ranges, and correlation coefficients (R^2) for the six ginsenosides in SLC determined by HPLC-DAD

Compounds	Calibration curve	Linear range (µg g^{-1})	Coefficient of determination (R^2)
Ginsenoside Rg1	y = 21.224x + 189.800	5.18 - 517.74	0.9984
Majonoside R2	y = 545.8x + 6,719.6	14.47 - 2,170.94	0.9996

Compounds	Calibration curve	Linear range (μg g^{-1})	Coefficient of determination (R^2)
Ginsenoside Rb1	y = 5.877x + 13.823	5.96 - 238.41	0.9999
Notoginsenoside R4	y = 17.511x + 76.715	5.19 - 207.76	0.9988
Notoginsenoside Fc	y = 13.161x + 31.301	4.81 - 192.23	0.9990
Ginsenoside Rd	y = 14.887x + 18.666	7.09 - 283.49	0.9993

3.3.4. LOD and LOQ

The LOD and LOQ of the six ginsenosides were estimated by performing seven replicate analyses and calculating the standard deviations of their concentrations. The results are presented in Table 4. The LOD values for the six ginsenosides in the SLC matrix ranged from 0.012% to 0.021%, while the LOQ values ranged from 0.040% to 0.068%. Signal-to-noise (S/N) ratios for all analytes were between 3 and 20, indicating that the concentrations used to estimate LOD and LOQ were appropriate and suitable for method validation.

Table 4. LOD and LOQ for six ginsenosides in SLC determined by HPLC-DAD (n = 7)

Compounds	G-Rg1	M-R2	G-Rb1	N-R4	N-Fc	G-Rd
LOD (%)	0.014	0.021	0.015	0.015	0.012	0.015
LOQ (%)	0.046	0.068	0.051	0.049	0.040	0.051
S/N	13.77	3.69	25.55	11.52	9.12	8.81

3.3.5. Precision

Precision of the developed HPLC-DAD method for simultaneous quantification of six ginsenosides in SLC was evaluated in terms of repeatability (intra-day precision) and reproducibility (inter-day precision), as summarized in Tables 5 and 6. The relative standard deviations for repeatability (RSDr) were below 2.5%, and those for reproducibility (RSDR) were below 4% for all six ginsenosides, indicating that the method meets the precision criteria according to AOAC 2016 Appendix F.

Table 5. Repeatability of six ginsenosides in SLC determined by HPLC-DAD

Repeatability (n = 7)	G-Rg1	M-R2	G-Rb1	N-R4	N-Fc	G-Rd
Average	3.92	4.91	1.52	0.65	0.41	0.49
RSD_r	1.01	0.92	2.36	1.77	1.94	2.25

Table 6. Reproducibility of six ginsenosides in SLC determined by HPLC-DAD

Reproducibility (n = 7)		G-Rg1	M-R2	G-Rb1	N-R4	N- Fc	G Rd
Day 1	**Average**	3.92	4.91	1.52	0.65	0.41	0.49
	RSD	1.01	0.92	2.36	1.77	1.94	2.25
Day 2	**Average**	3.91	4.91	1.56	0.66	0.41	0.49
	RSD	1.71	1.47	2.30	1.85	2.12	1.69
	Average	3.92	4.91	1.54	0.66	0.41	0.49
	RSD_R	1.98	1.74	3.30	2.56	2.87	2.81

3.3.6. Accuracy

The accuracy of the simultaneous quantification method for six ginsenosides in SLC is presented in Table 7. The initial ginsenoside contents in the test sample were 39.33 mg·g^{-1} for G-Rg1, 50.06 mg·g^{-1} for M-R2, 14.94 mg·g^{-1} for G-Rb1, 6.34 mg·g^{-1} for N-R4, 4.13 mg·g^{-1} for N-Fc, and 4.72 mg·g^{-1} for G-Rd. Spike recovery experiments were performed at three concentration levels (80%, 100%, and 120%) to evaluate method performance. The recoveries of all six ginsenosides ranged from 97% to 103%, meeting the criteria specified in AOAC 2016, Appendix F.

Table 7. Accuracy (recovery) of six ginsenosides in SLC determined by HPLC-DAD

Compounds	Recovery percentage (%)	Compounds	Recovery percentage (%)
Ginsenoside Rg1	97.74 - 102.67	Notoginsenoside R4	98.03 - 102.86
Majonoside R2	97.08 - 102.01	Notoginsenoside Fc	97.53 - 102.81
Ginsenoside Rb1	97.08 - 102.47	Ginsenoside Rd	97.27 - 102.89

4. DISCUSSION

In this study, a comprehensive optimization of extraction conditions and validation of an HPLC-DAD method were performed to enable the simultaneous quantification of six characteristic ginsenosides in SLC. The results collectively demonstrate that both extraction efficiency and analytical reliability are strongly influenced by solvent polarity, extraction parameters, and chromatographic conditions.

The comparison of 13 solvent systems clearly showed that EtOH:H_2O (1:1) provided the highest and most consistent yields for all six ginsenosides. This variation can be attributed to the distinct interactions between solvent polarity and the structural characteristics of ginsenosides. These compounds are triterpenoid saponin glycosides composed of a hydrophobic aglycone core and one or more hydrophilic sugar chains attached through O-glycosidic linkages at the C-3, C-6, or C-20 positions. Their amphiphilic nature enables the sugar moieties to form hydrogen bonds with polar solvents, while the aglycone interacts more effectively with less polar phases. As a result, mixed aqueous-alcohol systems such as EtOH:H_2O offer an optimal polarity range that enhances solubilization of both structural domains, thereby maximizing extraction yield. Optimization of operational parameters further supported efficient extraction: a solvent-to-sample ratio of 1/50 (w/v) and a 20-minute ultrasonic extraction ensured near-complete recovery, while two extraction cycles were sufficient to exhaust target analytes. These findings align with extraction behaviors reported for other *Panax* species [6 - 8].

Method validation confirmed that the HPLC-DAD procedure meets international analytical standards. Chromatographic separation achieved baseline resolution for all six ginsenosides, and system suitability parameters - including tR stability, peak symmetry, and theoretical plate numbers-fulfilled ICH requirements. The method demonstrated excellent specificity, with no interference observed in blank or samples.

Linearity was strong across all analytes ($R^2 > 0.998$), while LOD and LOQ values indicated adequate sensitivity for quantitative assessment of SLC. Precision testing showed low RSDr and RSDR values, meeting AOAC 2016 Appendix F criteria. Recovery experiments (97 - 103%) confirmed the method's accuracy and robustness across variable concentration levels.

Thus, the optimized extraction conditions and validated HPLC-DAD method provide a reliable analytical platform for routine quality assessment of ginsenosides in SLC. Differences observed across solvents and extraction parameters highlight the importance of appropriate method optimization when evaluating triterpenoid saponins in herbal.

5. CONCLUSION

In this study, an HPLC-DAD method was successfully developed and validated for the simultaneous quantification of six ginsenosides: Ginsenoside Rg1, Majonoside R2, Ginsenoside Rb1, Notoginsenoside R4, Notoginsenoside Fc, and Ginsenoside Rd - in SLC. The optimal extraction conditions were determined to be ethanol-water (1:1, v/v) as the extraction solvent, a sample-to-solvent ratio of 1:50 (w/v), ultrasonication for 20 minutes, and two extraction cycles. Chromatographic separation was achieved on a Shim-pack GIST C18 column (250 × 4.6 mm; 5 µm) at 50 °C using a gradient elution system of acetonitrile and water, with detection at 195 nm.

The method validation results met the ICH requirements, demonstrating good system suitability (RSD < 2%, Rs > 1.5, theoretical plates > 120,000), high specificity, excellent linearity (R^2 > 0.999) over suitable concentration ranges, satisfactory limits of detection (0.012-0.021%) and quantification (0.040-0.068%), and acceptable recovery values (97 - 103%) at three spiking levels.

Overall, the proposed HPLC-DAD method is reliable, accurate, and reproducible, making it suitable for the routine quality control and quantitative analysis of ginsenosides in SLC and related herbal preparations

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Xây dựng và thẩm định quy trình định lượng đồng thời sáu ginsenosid trong Sâm Lai Châu trồng bằng phương pháp HPLC-DAD

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TÓM TẮT

Đặt vấn đề: Sâm Lai Châu (Panax vietnamensis var. fuscidiscus K. Komatsu, S. Zhu & S.Q. Cai) là dược liệu có giá trị của Việt Nam, với thành phần hóa học chủ yếu là các ginsenosid có hoạt tính sinh học. Mục tiêu: Nghiên cứu này nhằm xây dựng và thẩm định phương pháp sắc ký lỏng hiệu năng cao kết hợp đầu dò diode (HPLC-DAD) để định lượng đồng thời sáu ginsenosid: ginsenosid Rg1, majonosid R2, ginsenosid Rb1, notoginsenosid R4, notoginsenosid Fc và ginsenosid Rd trong SLC. Phương pháp: Sử dụng cột Shim-pack GIST C18 (250 × 4.6 mm, 5 µm) trên hệ thống Shimadzu LC-2030C 3D Plus, chương trình rửa giải gradient với pha động là nước và acetonitril. Phát hiện tại bước sóng 195 nm, sáu hợp chất được tách hoàn toàn trong 50 phút (Rs > 1.5).Kết quả: Phương pháp định lượng đồng thời sáu ginsenosid trong SLC được thẩm định có độ đặc hiệu cao, tuyến tính tốt (R^2 > 0.999) và độ chính xác lặp lại cao (RSD < 2.7%). Giới hạn phát hiện (LOD) dao động từ 0.012% đến 0.021% và giới hạn định lượng (LOQ) từ 0.040% đến 0.068%. Độ thu hồi dao động từ 97.08% đến 102.89%, chứng tỏ độ đúng của phương pháp. Kết luận: Phương pháp HPLC-DAD được xây dựng và thẩm định cho thấy có độ tin cậy cao, phù hợp cho việc kiểm nghiệm dược liệu SLC cũng như các chế phẩm từ Sâm.

***Từ khóa**: Sâm Lai Châu, Panax vietnamensis var. fuscidiscus, HPLC-DAD*

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