

Quantification of major saponins in *Panax vietnamensis* by HPLC-PDA via quantitative analysis of multicomponent by a single marker

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ABSTRACT

Background: *Panax vietnamensis* (Vietnamese ginseng) is an endemic medicinal plant that contains high levels of ootillol-type saponins, mainly majonoside R2. The quality of this plant is important for its medicinal use and product development. However, conventional quantification methods that use several reference standards are expensive for routine analysis. **Objectives:** This study aimed to develop a simple method for the quantitative analysis of multiple saponins using ginsenoside Rb₁ as a single reference standard, and to evaluate the accuracy of this new method by comparing it with the external standard method (ESM). **Subjects and methods:** The quantitative analysis of multi-components by a single marker (QAMS) method was used to quantify four ginsenosides (G-Rb₁, G-Rd, G-Rg₁, and M-R2) with G-Rb₁ as a single reference standard. Relative conversion factors (F_i) and relative retention times (RRT) were applied, and differences between QAMS and ESM results were compared using %SMD. **Results:** The slope-based F_i values (n = 3) were 1.000 (G-Rb₁), 1.087 (G-Rd), 1.317 (G-Rg₁), and 0.037 (M-R2). The QAMS method met all validation criteria, and the quantitative results were highly consistent with those obtained by ESM, with %SMD values below 5.00% for all saponins analyzed. **Conclusions:** This study successfully developed and validated a QAMS method using G-Rb₁ as a single reference standard for quantifying major saponins in *Panax vietnamensis*. The method provides accurate, reliable, and cost-effective results comparable to the traditional external standard method. QAMS has been increasingly adopted in advanced pharmacopoeias worldwide, reflecting a global trend toward more efficient and practical analytical methods. By introducing this modern approach for Vietnamese ginseng, the study reduces dependence on multiple reference standards, lowers testing costs, and provides a reliable tool for routine analysis and standardization.

Keywords: *Panax vietnamensis*, HPLC, QAMS, quantitative analysis, saponin

1. INTRODUCTION

Panax vietnamensis Ha et Grushv., known as Vietnamese ginseng, is an endemic species of Vietnam. It is considered a “national treasure” because of its rarity and high medicinal value. Studies have identified more than 52 types of saponins in this species. The main ones include ginsenoside Rb₁ (G-Rb₁), ginsenoside Rd (G-Rd), ginsenoside Rg₁ (G-Rg₁), and ootillol-type saponins such as majonoside R2 (M-R2). These compounds contribute to many biological effects, including antioxidant, hepatoprotective, anti-stress, and neuroprotective activities [1]. The use of Vietnamese ginseng in herbal medicine and health supplements is growing. Therefore, reliable analytical methods are needed to ensure the quality, efficacy, and safety of raw materials and products. These methods must be able to determine the content of major saponins accurately.

Advanced chromatographic techniques such as high-performance liquid chromatography (HPLC) combined with ultraviolet (UV), photodiode array (PDA), or diode array (DAD) detectors are widely used in research and pharmacopoeias to quantify saponins in *Panax* species [2]. Species in the *Panax* genus have very similar chemical compositions. Therefore, simultaneous quantification of multiple saponins is necessary for accurate identification and reliable quality control. In the European Pharmacopoeia (EP 11.0), an HPLC-UV method is used to determine G-Rg₁, G-Rb₁, and G-Rf simultaneously in *Panax notoginseng* [3, 4]. The USP47-NF42 on American Ginseng Root and Rhizome monograph requires several reference standards, including G-Rg₁, G-Re, G-Rb₁, G-Rc, G-Rd, and G-Rb₂ [3]. Similarly, the Vietnamese Pharmacopoeia V recommends G-Rg₁, G-Rb₁, M-R2, and G-Rd as reference markers for

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Vietnamese ginseng [5]. These findings show that HPLC-UV is simple, cost-effective, and suitable for quality control of herbal. However, the use of multiple external reference standards increases testing costs, creates dependency on suppliers, and causes delays in purchasing and standard preparation.

To overcome these limitations, researchers developed the quantitative analysis of multiple components by a single marker (QAMS) method. This approach uses one reference standard to simultaneously determine the concentrations of multi-components. It helps reduce cost, shorten analysis time, and improve practicality. In the study by Jia et al., the contents of 25 compounds were determined using the QAMS method with G-Rb₂ as a single reference standard [6]. Similarly, a study on *P. notoginseng* used G-Re as the single marker to accurately quantify N-R1, G-Rg₁, Rb₁, and Rd [7]. Both studies showed no significant differences between QAMS and the external standard method (ESM), confirming that QAMS is a reliable and accurate approach for determining component concentrations.

This study aimed to: (1) develop and validate a simple, accurate, and low-cost HPLC-PDA method combined with QAMS for the simultaneous analysis of major saponins in *Panax vietnamensis*; (2) apply the method to evaluate the quality of Vietnamese ginseng; and (3) verify the reliability of QAMS by comparing it with the external standard method (ESM).

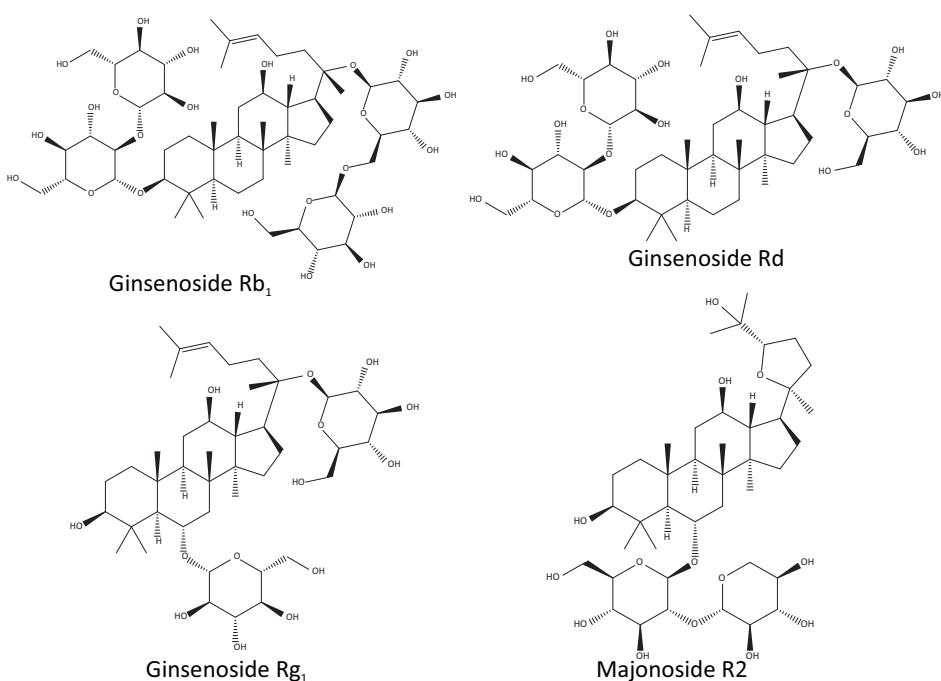


Figure 1. Chemical structures of the reference standards

2. MATERIALS AND METHODS

2.1. Plant materials and standards

Rhizomes and roots of *Panax vietnamensis* were collected from the Tra Linh Ginseng Farm in Nam Tra My District, Quang Nam Province, Vietnam. The plant materials were authenticated according to the quality evaluation criteria of the Vietnamese Pharmacopoeia V, then dried at 60°C, powdered to a particle size of < 1 mm, and stored for analysis.

Reference standards of G-Rb₁, G-Rd, G-Rg₁, and M-R2 (purity > 94%) were obtained from the University of Medicine and Pharmacy, Ho Chi Minh City.

2.2. Sample preparation

An exact amount of 100 mg of Vietnamese ginseng powder was extracted three times with 5 mL of 70% methanol under ultrasonication (20 min each). The combined extracts were centrifuged, evaporated to dryness in a water bath at 100°C for 30 minutes, and then redissolved in water. They were subsequently loaded onto a C18 SPE cartridge that had been preconditioned with methanol and water. After washing with water to remove polar impurities, the saponins were eluted with methanol containing 1% formic acid, collected in a 5 mL volumetric flask, and filtered through a 0.45 µm membrane before injection [1].

Standard preparation: A mixed standard solution (C1) of G-Rb₁, G-Rd, G-Rg₁, M-R2, was prepared in 100% methanol at concentrations of 0.357 mg/mL, 0.172 mg/mL, 1.385 mg/mL, 2.300 mg/mL, and 0.810 mg/mL, respectively. This solution was serially diluted with 100% methanol using a dilution factor of 0.75 to obtain C2-C6 standard mixtures for calibration curve establishment [2].

2.2.1. Establishment and validation of QAMS method

Selection of the single reference standard for QAMS

In the QAMS method, the aim is to quantify multiple saponins while using only one reference standard. For *Panax vietnamensis*, the major saponins to be quantified are ginsenoside Rg₁, ginsenoside Rb₁, ginsenoside Rd, and majonoside R2. Therefore, one of these compounds must be selected as the single reference standard to calculate the relative conversion factors for the others. The selected reference standard should have a high and stable content in the sample, present a well-resolved and easily identifiable chromatographic peak, and be available as a certified reference standard for accurate calibration. In addition, it should exhibit a stable and linear detector response to ensure reliable calculation of conversion factors (F_i) in the QAMS analysis [8].

Calculation of relative retention times and relative conversion factors

The relative conversion factor (F_i) is defined as the ratio of the response factor of each analyte to that of the single reference standard in QAMS. Meanwhile, the relative retention time (RRT_i) represents the ratio between the retention time of an analyte and that of the reference standard, serving to identify chromatographic peaks. There are several methods for determining relative conversion factors (F_i); however, in this study, the slope of the calibration curve was employed, as it makes use of the regression line from all calibration points, thereby reducing variability, minimizing the influence of concentration differences, and ensuring more stable and reliable relative conversion factors [9].

$$F_i = \frac{Slope_i}{Slope_s} \quad (1)$$

$$RRT_i = \frac{R_i}{R_s} \quad (2)$$

$$C_i = \frac{(A_i - b_i) \times C_s}{(A_s - b_s) \times F_i} \quad (3)$$

The relative conversion factors for each analyte (F_i) were calculated from the calibration curves of the reference standards according to Equation (1), where $Slope_i$ represents the slope of the calibration curve of the target compound ($G\text{-Rg}_1$,

$G\text{-Rd}$, or $M\text{-R2}$), and $Slope_s$ denotes the slope of the calibration curve of the internal standard $G\text{-Rb}_1$. Relative retention times (RRT_i) were determined using Equation (2), where R_s represents the retention time of the internal standard ($G\text{-Rb}_1$) and R_i represents the retention time of the target analyte (in minutes). The concentration of each analyte (C_i) was then calculated using Equation (3), where A_i and A_s are the peak areas of the analyte and the internal standard (mAU^*s), C_i and C_s are their respective concentrations (mg/mL), and b_i and b_s are the intercepts of the corresponding regression equations.

Analytical method validation

The experiments were conducted using a Shimadzu HPLC-PDA Prominence-I LC-2030C 3D Plus system. The Phenomenex Luna C18 chromatography column (150 mm × 4.6 mm, 5 μm) was used to separate the saponins. The injection volume was set to 25 μL , with a flow rate of 0.8 mL/min , and the column temperature was maintained at 30°C. The mobile phase consists of acetonitrile (A) and water (B) with the following gradient program: 0 to 25 min, 23% A; 25 to 35 min, 23% to 32% A; 35 to 50 min, 32% A; 50 to 68 min, 32% to 35% A, 68 to 75 min, 35% to 95% A. After a 10-minute equilibration period, the samples were injected. The detection wavelength was set at 196 nm for PDA.

The HPLC method was validated according to ICH and AOAC guidelines, assessing specificity, linearity, precision, and accuracy [10, 11].

2.2.2. Method applications

The major saponins of *Panax vietnamensis*, including $G\text{-Rg}_1$, $G\text{-Rb}_1$, $G\text{-Rd}$, and $M\text{-R2}$, were quantified using both QAMS and the conventional external standard method (ESM). The external standard method is a routine quantitative approach widely used in research and official pharmacopeias worldwide. In this study, the standard method difference (%SMD) represents the percentage difference between the results obtained by the QAMS and ESM methods. The smaller the %SMD value, the less difference there is between the two methods, confirming the reliability of QAMS for the quality evaluation of *Panax vietnamensis*. % SMD was calculated

according to Equation (4).

$$SMD (\%) = \frac{|H_{ESM} - H_{QAMS}|}{H_{ESM}} \times 100 \quad (4)$$

Where H_{ESM} and H_{QAMS} represent the content of saponin in an analyte assayed by the ESM and QAMS methods, respectively.



Figure 2. Rhizomes and roots of *Panax vietnamensis*

3.2. Selection of the single reference standard for QAMS

Ginsenoside Rb_1 was selected as the internal standard for several reasons. First, it belongs to the protopanaxadiol (PPD) group, which is one of the principal saponin classes in *Panax vietnamensis*, and it exhibits the highest extraction yield within this group (approximately 2.0%), thereby ensuring that a sufficient amount can be readily obtained [12]. Second, $G-Rb_1$ can be obtained not only from *P. vietnamensis* but also from other *Panax* species, making it more accessible and economical compared to the rare ootillol-type saponins. Third, it demonstrates high chemical stability

3. RESULTS

3.1. Plant materials

The roots of *P. vietnamensis* were washed, sliced, and dried at 60°C. After drying, the herbal materials were ground into a fine, homogeneous powder and passed through a 1 mm sieve. The powder was then tested and met the requirements of the "Vietnamese Ginseng" monograph in the Vietnamese Pharmacopoeia V.

under chromatographic conditions and produces distinct, well-resolved peaks. In addition, commercial reference standards of $G-Rb_1$ with certified high purity are widely available, which enables reliable calibration and consistent determination of relative conversion factors. These advantages make $G-Rb_1$ a representative and practical choice as the internal standard in QAMS analysis of *P. vietnamensis*.

3.3. Calculation of relative retention times and relative conversion factors

In this study, ginsenoside Rb_1 was employed as the single reference standard ($F_i = 1.000$), and the

relative conversion factors (F_i) of the other analytes were calculated based on the slope ratios of calibration curves obtained on three separate days. The mean F_i values were 1.317 for G-Rg₁,

0.037 for M-R2, and 1.087 for G-Rd (Table 1). The %RSD values ranged from 3.15% to 5.68%, indicating that the F_i values were stable across different runs.

Table 1. The average relative conversion factors (F_i) of 4 ginsenosides

Day	Average relative conversion factors (F_i) (n = 3)			
	G-Rg ₁	M-R2	G-Rb ₁	G-Rd
1	1.311	0.035	1.000	1.028
2	1.279	0.039	1.000	1.104
3	1.361	0.036	1.000	1.130
Mean \pm SD	1.317 \pm 0.042	0.037 \pm 0.002	1.000 \pm 0.000	1.087 \pm 0.053
%RSD	3.15	5.68	0.00	4.87

Accurate peak identification is essential for QAMS analysis. In this study, the relative retention times (RRT_i) of ginsenosides were calculated as the ratio of the retention time of each analyte to that of the single reference standard ginsenoside Rb₁. The mean RRT_i values were 0.286 for G-Rg₁, 0.341 for

M-R2, and 1.342 for G-Rd, with %RSD values ranging from 0.00% to 0.23% (Table 2). These low %RSD values demonstrate that the RRT_i were highly stable across three consecutive days, confirming their reproducibility under the tested chromatographic conditions.

Table 2. The average relative retention times of 4 ginsenosides

Day	Average relative retention times (RRT _i) (n = 3)			
	G-Rg ₁	M-R2	G-Rb ₁	G-Rd
1	0.286	0.341	1.000	1.340
2	0.286	0.341	1.000	1.343
3	0.285	0.340	1.000	1.342
Mean \pm SD	0.286 \pm 0.001	0.341 \pm 0.001	1.000 \pm 0.000	1.342 \pm 0.001
%RSD	0.21	0.23	0.00	0.09

3.4. Analytical method validation

Specificity was evaluated by comparing chromatograms of the blank, standard, sample, and sample solutions spiked with reference standards under identical HPLC conditions. The retention times of the investigated saponins in the standard and sample solutions were consistent. No interfering peaks were detected at the corresponding retention times in the blank chromatogram, confirming the absence of matrix interference. Furthermore, in the spiked sample, the peak areas of the major saponins increased

proportionally compared to those in the unspiked sample, demonstrating the method's ability to identify the analytes accurately. These findings indicate that the developed HPLC-PDA method possesses adequate specificity for the quantitative determination of saponins in *Panax vietnamensis*.

The linear regression equations and corresponding linear ranges for the quantified saponins are presented in Tables 3 and 4, demonstrating good linearity within the tested concentration ranges, with correlation coefficients (R^2) ranging from 0.9996 to 0.9999.

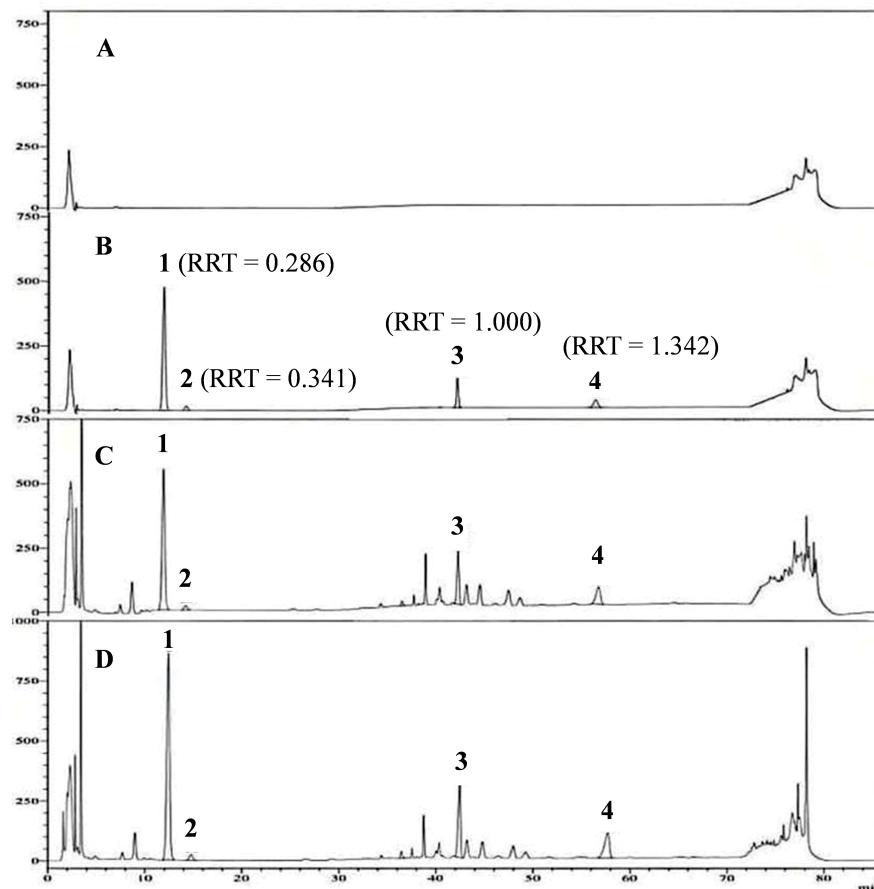
Table 3. Concentration and peak area of standard solution series

No.	G-Rg ₁		M-R2		G-Rb ₁		G-Rd	
	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)
1	1.408	19631027	2.302	875142	0.357	3684627	0.180	2006309

No.	G-Rg ₁		M-R2		G-Rb ₁		G-Rd	
	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)	C (mg/mL)	S (mAU*s)
2	1.056	15105443	1.726	659631	0.268	2753371	0.135	1510452
3	0.792	11599512	1.295	495199	0.201	2073976	0.101	1129896
4	0.594	8915424	0.971	379730	0.151	1575932	0.076	856396
5	0.446	6806252	0.728	286324	0.113	1190326	0.057	645844
6	0.334	5189898	0.546	218091	0.085	904171	0.043	491770

Table 4. Results of the investigation of linearity, linear range

Component	Linear regression equation (n = 3)	R ²	Linear range (mg/mL)
G-Rg ₁	y = 13425651 x + 846186	0.9996	0.334 - 1.408
M-R2	y = 373839 x + 14146	0.9999	0.546 - 2.302
G-Rb ₁	y = 10175363 x + 50838	0.9998	0.085 - 0.357
G-Rd	y = 11034568 x + 17292	0.9999	0.043 - 0.180

**Figure 3.** Specificity of HPLC-PDA method illustrated by chromatograms of (A) blank, (B) standard, (C) sample, and (D) spiked sample (1. G-Rg₁, 2. M-R2, 3. G-Rb₁, 4. G-Rd)

Repeatability tests using HPLC-PDA yielded %RSD values of 0.88% - 1.62%, demonstrating the method's good consistency. Reproducibility was further assessed on two independent HPLC

systems. The quantification of G-Rb₁, G-Rg₁, and M-R2 yielded consistent results, with RSD values ranging from 1.89% to 2.86%. In contrast, G-Rd exhibited higher variability (%RSD = 6.47%), which

can be attributed to its relatively low content and weak absorbance signal, resulting in greater fluctuations between instruments. Accuracy was evaluated using the standard addition method at three concentration levels (approximately 80%, 100%, and 120% of the nominal content), each analyzed in triplicate. The mean recoveries for the

four analytes ranged from 79.37% to 108.45%, confirming that the HPLC-PDA method ensures reliable precision and accuracy for the quantitative determination of saponins in *Panax vietnamensis*. Detailed results of precision, reproducibility, and recovery are summarized in Table 5.

Table 5. Results of the investigation of precision

Compound	Repeatability (%RSD) (n = 6)	Reproducibility (%RSD) (n = 12)	Recovery (%) (n = 9)
G-Rg ₁	0.88	2.86	85.98 - 108.45
M-R2	0.96	1.94	81.75 - 99.90
G-Rb ₁	1.10	1.89	80.04 - 95.30
G-Rd	1.62	6.47	79.37 - 97.46

3.5. Method applications

In the present study, the quantitative results obtained by QAMS were compared with those from the external standard method (ESM). As presented in Table 6, the contents of G-Rg₁, M-R2, G-Rb₁, and G-Rd determined by both methods were highly consistent, with mean values and standard deviations showing no significant

differences. The calculated SMD values ranged from 0.00% to 0.11%, all well below the recommended threshold of 5% [13]. These findings demonstrate that the QAMS method, using G-Rb₁ as the single reference standard, provides accuracy and reliability comparable to the conventional ESM approach for quantifying saponins in *Panax vietnamensis*.

Table 6. Saponin content and SMD, determined by HPLC-PDA (average F_i, n = 3)

Method	No.	The mean saponin content was calculated on a dried herb (%) with F _i values according to G-Rb ₁			
		G-Rg ₁	M-R2	G-Rb ₁	G-Rd
ESM	1	3.80	4.77	1.51	1.05
	2	3.79	5.01	1.46	0.99
	3	3.76	4.79	1.49	0.97
	Mean ± SD	3.78 ± 0.02	4.86 ± 0.13	1.49 ± 0.03	1.00 ± 0.04
QAMS	1	3.80	4.78	1.51	1.05
	2	3.59	5.02	1.46	0.99
	3	3.76	4.80	1.49	0.97
	Mean ± SD	3.78 ± 0.02	4.86 ± 0.13	1.49 ± 0.03	1.00 ± 0.04
% SMD	Mean ± SD	0.01 ± 0.00	0.11 ± 0.00	0.00 ± 0.00	0.01 ± 0.00

4. DISCUSSION

Ocotillol-type saponins, such as M-R2, contain few or no chromophores in their molecular structures. As a result, they exhibit very weak UV absorption, making detection by photodiode array (PDA) detectors challenging. However, M-R2 can be effectively detected and quantified at 196 nm with good sensitivity and excellent linearity, despite the absence of a double bond in its aglycone [14]. This wavelength provided a markedly stronger

absorption response than 198 or 203 nm, enabling reliable quantification by UV detection. Moreover, UV or PDA detectors remain widely available, cost-effective, and straightforward to implement in routine analytical laboratories.

Notably, the Vietnamese Pharmacopoeia V also specifies UV detection for the assay of major saponins in *Panax vietnamensis*, further supporting the continued use of UV-based

detection at 196 nm as a practical and validated approach for M-R2. Consistent with these findings, the present study achieved an excellent linear correlation for M-R2 using HPLC-PDA detection at 196 nm, with a determination coefficient (R^2) of 0.9999, confirming the method's high sensitivity and reliable linearity across the tested concentration range.

The choice of a single reference standard affects the accuracy and reliability of QAMS. Ginsenoside Rb₁ was selected for several reasons. It occurs in many *Panax* species, allowing easy isolation and purification while providing a stable and feasible supply. Moreover, it exists in high amounts in *P. vietnamensis*, enabling precise detection and accurate quantification. Additionally, G-Rb₁ remains chemically stable and undergoes minimal degradation during analysis, ensuring reliable results. Therefore, using G-Rb₁ as a single reference standard determines the main saponins in Vietnamese ginseng accurately. Finally, this approach simplifies the procedure, reduces the need for multiple standards, and minimizes potential errors.

Average relative conversion factors (F_i), calculated from slope ratios of calibration curves across three different days, were consistent with %RSD values ranging from 3.15% to 5.68%, confirming their reproducibility. Similarly, the relative retention times (RRT_i) of the analytes to G-Rb₁ showed minimal variation (%RSD \leq 0.23%), ensuring accurate peak recognition and reducing the risk of misidentification in complex chromatographic profiles.

The results showed that the QAMS method using ginsenoside Rb₁ as the single reference standard had accuracy equivalent to the ESM method. The %SMD values ranged from 0.00 to 0.11%, which is far below the acceptable limit of 5%. This finding is consistent with previous studies applying QAMS to saponins in the genus *Panax*. For example, Jia et al. quantified 25 ginsenosides and reported SMD values of 0.1 - 5.45%, indicating that QAMS can replace ESM when reference standards are limited [6]. Li et al. also showed that a single marker could be used to quantify multiple ginsenosides in *Panax notoginseng* [15]. Meanwhile, Stekolshchikova et al. applied QAMS to PPD-type, PPT-type, and OT-type ginsenosides using HPLC-MS. Due to variation

in F values, the authors selected group reference standards, using G-Rb₁ for PPD-type and pseudoginsenoside F11 for OT-type saponins [16]. In contrast, our study is the first to demonstrate that QAMS can be used to quantify M-R2, an OT-type saponin characteristic of *Panax vietnamensis*, without requiring a separate reference standard for the OT-type group. This is important because purified M-R2 is scarce and expensive in Vietnam, which makes routine analysis difficult. Although M-R2 shows weak UV absorption, detection at 196 nm still provided stable and reliable signals. The difference between the results of QAMS and ESM methods was only \sim 0.11%, confirming the accuracy of the method. Therefore, G-Rb₁ can be used as a single reference standard to quantify PPD-, PPT-, and OT-type saponins simultaneously in *Panax vietnamensis*. This approach reduces analytical cost, simplifies laboratory workflow, and provides a scientific basis for the potential inclusion of QAMS in the Vietnamese Pharmacopoeia.

5. CONCLUSION

This study successfully established a QAMS strategy for the simultaneous quantification of major saponins in *Panax vietnamensis* using ginsenoside Rb₁ as the internal reference. The method showed stable F_i and RRT_i values and high agreement with the ESM method, indicating reliable accuracy. Using a single reference standard reduced analytical cost and simplified routine laboratory procedures. Therefore, QAMS is a practical and efficient approach for the quality control of *P. vietnamensis*.

However, the QAMS method still has several limitations. OT-type saponins such as M-R2 have very weak UV absorption, so detection at 196 nm requires careful control of the baseline and solvent purity to avoid signal interference. It is also necessary to adjust the sample concentration to ensure that the M-R2 signal remains above the LOQ for reliable quantification. In addition, the method should be validated using raw materials from different cultivation regions and across various processed forms and product types to confirm its robustness in routine quality control. In future studies, QAMS may be combined with detectors that do not depend on UV absorption,

such as ELSD, CAD, or MS. These detectors may improve the sensitivity and accuracy in quantifying OT-type saponins in *Panax vietnamensis*.

REFERENCES

[1] K. L. Vu-Huynh *et al.*, "Accumulation of Saponins in Underground Parts of *Panax vietnamensis* at Different Ages Analyzed by HPLC-UV/ELSD," *Molecules*, vol. 25, no. 13, p. 3086, 2020. <https://doi.org/10.3390/molecules25133086>.

[2] A. Wang, C. Z. Wang, J. A. Wu, J. Osinski, and C. S. Yuan, "Determination of major ginsenosides in *Panax quinquefolius* (American ginseng) using high-performance liquid chromatography," (in eng), *Phytochem Anal*, vol. 16, no. 4, pp. 272-7, Jul-Aug 2005. <https://doi.org/10.1002/pca.838>.

[3] The United States Pharmacopoeial Convention, *United States Pharmacopeia and National Formulary (USP 2024)*, Rockville, MD, USA: United States Pharmacopoeial Convention, 2024.

[4] European Directorate for the Quality of Medicines & HealthCare (EDQM), *European Pharmacopoeia (Ph. Eur.) Supplement 11.2*, Strasbourg, France: Council of Europe, 2024.

[5] Ministry of Health (Vietnam), *Vietnamese Pharmacopoeia V*, Hanoi: Medical Publishing House, 2017.

[6] X. Jia, C. Hu, X. Zhu, Y. Yuan, and Y. Zhou, "Simultaneous Determination of 25 Ginsenosides by UPLC-HRMS via Quantitative Analysis of Multicomponents by Single Marker," *International Journal of Analytical Chemistry*, vol. 2021, pp. 1-11, 07/01 2021. <https://doi.org/10.1155/2021/9986793>.

[7] D. Y. Wen-jie, "Determination of five saponins in extract of notoginseng total saponins by quantitative analysis of multi-components by single marker," *Chinese Journal of Pharmaceutical Analysis*, vol. 42, no. 3, pp. 518-524, 2022.

[8] K. Abudureheman, Q. Wang, H. Zhang, and X. Gong, "Development of a QAMS Analysis Method for Industrial Lanolin Alcohol Based on the Concept of Analytical Quality by Design," *Separations*, vol. 11, no. 9, p. 276, 2024. <https://doi.org/10.3390/separations11090276>.

[9] J. Da *et al.*, "A reproducible analytical system based on the multi-component analysis of triterpene acids in *Ganoderma lucidum*," (in eng), *Phytochemistry*, vol. 114, pp. 146-54, Jun 2015. <https://doi.org/10.1016/j.phytochem.2014.08.007>.

[10] AOAC International, *Appendix K: Guidelines for Dietary Supplements and Botanicals*, Rockville, MD, USA: AOAC International, 2023.

[11] International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use, *ICH Q2(R2): Validation of Analytical Procedures*, Geneva: ICH, 2024.

[12] N. T. H. Tran Bao Tram, Pham Huong Son, Pham The Hai, "Evaluation of growth and bioactive compositions of Vietnamese ginseng (*Panax vietnamensis*) cultivated in Quang Nam," *Natural sciences and technology*, vol. 33, no. 2S, 2017. <https://doi.org/10.25073/2588-1140/vnunst.4594>.

[13] Y. Li *et al.*, "Quantitative analysis of multi-components by single marker method combined with UPLC-PAD fingerprint analysis based on saikosaponin for discrimination of Bupleuri Radix according to geographical origin," (in eng), *Original Research*, vol. 11, January-19 2024. <https://doi.org/10.3389/fchem.2023.1309965>.

[14] S. Zhu, K. Zou, S. Cai, M. R. Meselhy, and K. Komatsu, "Simultaneous determination of triterpene saponins in ginseng drugs by high-performance liquid chromatography," (in eng), *Chem Pharm Bull (Tokyo)*, vol. 52, no. 8, pp. 995-8, Aug 2004. <https://doi.org/10.1248/cpb.52.995>.

[15] S. P. Li *et al.*, "A novel strategy with standardized reference extract qualification and single compound quantitative evaluation for quality control of Panax notoginseng used as a functional food," (in eng), *J Chromatogr A*, vol. 1313, pp. 302-7, Oct 25 2013. <https://doi.org/10.1016/j.chroma.2013.07.025>.

[16] E. Stekolshchikova, P. Turova, O. Shpigun, I. Rodin, and A. Stavrianidi, "Application of quantitative analysis of multi-component system approach for determination of ginsenosides in different mass-spectrometric conditions," (in eng), *J Chromatogr A*, vol. 1574, pp. 82-90, Nov 2 2018. <https://doi.org/10.1016/j.chroma.2018.09.005>.

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Định lượng saponin chính trong *Panax vietnamensis* bằng HPLC-PDA theo phương pháp định lượng đa thành phần dùng một chuẩn

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TÓM TẮT

Đặt vấn đề: *Panax vietnamensis* (Sâm Việt Nam) là dược liệu đặc hữu của Việt Nam, chứa hàm lượng cao saponin khung ocotillol, tiêu biểu là majonoside R2. Việc kiểm soát chất lượng dược liệu này rất quan trọng cho mục tiêu sử dụng và phát triển các chế phẩm từ sâm. Tuy nhiên, các phương pháp định lượng truyền thống đòi hỏi sử dụng nhiều chất chuẩn, gây tốn kém và khó áp dụng trong kiểm nghiệm thường quy. Mục tiêu nghiên cứu: Nghiên cứu này nhằm xây dựng phương pháp định lượng đồng thời nhiều saponin chỉ sử dụng một chất chuẩn duy nhất (ginsenoside Rb₁), đồng thời đánh giá độ chính xác của phương pháp thông qua so sánh với phương pháp chuẩn ngoại (ESM). **Đối tượng và phương pháp nghiên cứu:** Phương pháp định lượng đa thành phần bằng một chất chuẩn duy nhất (QAMS) được áp dụng để định lượng bốn saponin: G-Rb₁, G-Rd, G-Rg, và M-R2, sử dụng G-Rb₁ làm chất chuẩn đơn duy nhất. Hệ số chuyển đổi tương đối (F_i) và thời gian lưu tương đối (RRT) được xác định. Độ sai khác giữa trong kết quả định lượng bằng phương pháp QAMS và ESM được đánh giá thông qua giá trị %SMD. Kết quả: Giá trị F_i (n = 3) lần lượt là 1.0000 (G-Rb₁), 1.087 (G-Rd), 1.317 (G-Rg₁) và 0.037 (M-R2). QAMS cho kết quả định lượng có mức độ tương đồng cao với ESM, với SMD < 5.00% cho tất cả các saponin. Kết luận: Nghiên cứu đã xây dựng và thẩm định thành công phương pháp QAMS sử dụng G-Rb₁ làm chất chuẩn duy nhất trong định lượng các saponin chính của Sâm Việt Nam. Phương pháp cho kết quả chính xác, tương đương với phương pháp dùng hỗn hợp chuẩn ngoại truyền thống. QAMS hiện đang được áp dụng rộng rãi trong nhiều dược điển tiên tiến, thể hiện xu hướng toàn cầu hướng tới các phương pháp phân tích hiệu quả và thực tiễn hơn. Việc áp dụng phương pháp này cho sâm Việt Nam giúp giảm phụ thuộc vào nhiều chất chuẩn đắt tiền, hạ chi phí kiểm nghiệm, và cung cấp công cụ tin cậy cho chuẩn hóa và kiểm soát chất lượng thường quy.

Từ khóa: *Panax vietnamensis*, HPLC, QAMS, định lượng, saponin

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