Original article

Development and validation of an HPLC method for the quantification of strychnine in *Strychnos wallichiana* Steud. ex DC.

Nguyen Viet Khan[1*], Truong Tieu Bang[1], Le Anh Khoa[1], Chu Thi Huong[2], Dao Thi Cam Minh[1]

[1]Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University

[2]The college of Hue

***Corresponding author:** Nguyen Viet Khan; email: nvkhan@huemed-univ.edu.vn

Received: 21/01/2026; **Accepted:** 25/04/2026; **Published:** 30/04/2026

DOI: 10.34071/jmp.2026.2.922

Abstract

Background: *Strychnos wallichiana* Steud. ex DC. is a medicinal plant, in which strychnine exhibits strong biological activity but also carries high toxicity. Accurate quantification of strychnine in this medicinal plant is essential to ensure safe usage, particularly in research purposes and medical applications. The objective of this study was to develop and validate a method for the quantification of strychnine in *S. wallichiana* using HPLC.

Materials and methods: *S. wallichiana* samples were collected from Nghe An province. Strychnine was extracted from the plant material and analyzed by HPLC. Method validation was performed in accordance with analytical guidelines.

Results: The chromatographic conditions include an acetonitrile:buffer system (H_3PO_4 0.3% + $(C_2H_5)_3N$ 0.2%, v/v) in a 12:88 ratio (v/v) and a flow rate of 0.5 mL/min on an InertSustain™ C18 column (4.6 x 250 mm; 5 µm), and a detection wavelength of 263 nm. A linear response was obtained over the concentration range of 40 - 800 µg/mL for strychnine, with LOD and LOQ values of 0.3 µg/mL and 1.0 µg/mL, respectively. The analytical method demonstrated system suitability and high specificity, with a wide linear range and reliable accuracy and precision.

Conclusion: The developed HPLC method meets the required standards for the quantification of strychnine in *S. wallichiana* medicinal plant material.

Keywords: *strychnine, HPLC, validation, S. wallichiana, medicinal plant.*

1. INTRODUCTION

Strychnos wallichiana Steud. ex DC. (*S. wallichiana*), belonging to the family Loganiaceae, is a medicinal plant species distributed mainly in tropical regions of Asia, including India, Bangladesh, and Vietnam. In traditional medicine, various parts of the plant, particularly the roots and stem bark, have been used to treat a wide range of diseases such as rheumatism, musculoskeletal pain, gastrointestinal disorders, and certain skin ailments. Pharmacological studies have shown that the major chemical constituents of this species are indole alkaloids, among which strychnine and brucine are the principal compounds, exhibiting strong biological activities but also posing a high risk of toxicity [1-3].

Strychnine is a toxic alkaloid that can induce skeletal muscle convulsions and cause death due to respiratory failure when taken in excessive doses. Nevertheless, strychnine belongs to a class of compounds acting on the central nervous system and, at very low doses, has been used as a neuro- and neuromuscular stimulant. In classical medicine, it was applied as an adjunct therapy for conditions such as nervous exhaustion, mild limb paralysis, urinary dysfunction associated with reduced bladder smooth muscle tone, decreased intestinal motility, erectile dysfunction, and post-illness weakness [3–4]. Therefore, accurate quantification of strychnine in *S. wallichiana* is essential to ensure both safety and efficacy in therapeutic use and scientific research.

Several studies have applied modern chromatographic techniques to quantify strychnine in species of the genus *Strychnos*, among which reversed-phase high-performance liquid chromatography (RP-HPLC) is the most commonly employed method [3]. Other analytical approaches, including gas chromatography–mass spectrometry (GC-MS), capillary electrophoresis (CE), and high-performance thin-layer chromatography (HPTLC), have also been reported for the qualitative and quantitative analysis of alkaloids in *Strychnos* species. However, to date, no studies have reported the quantitative determination of strychnine in *S. wallichiana* in Vietnam, and such investigations remain limited worldwide [4,5].

Accordingly, the development of a standardized

and validated method for the quantification of strychnine in the plant is of significant importance. The objective of this study was to develop and validate an analytical method for the determination of strychnine in *S. wallichiana*. The results are expected to contribute to the improvement of quality control procedures for this medicinal material by ensuring that strychnine content is maintained within acceptable safety limits. This approach enhances user safety and the quality of herbal products, supports accurate dosing in traditional medicine practice, reduces poisoning risk, and promotes the sustainable use of this valuable medicinal resource.

2. MATERIALS AND METHODS

2.1. Materials, Chemicals, Solvents, and Instruments

Materials

The medicinal material used in this study was the stem bark of *S. wallichiana*. A total of 1.0 kg of dried material was collected from a traditional medicine pharmacy in Nghe An Province, Vietnam. The plant material was taxonomically authenticated by Dr. Le Tuan Anh (Mientrung Institute for Scientific Research, Vietnam). A voucher specimen (code: SW-01) has been deposited at the Faculty of Pharmacy, University of Medicine and Pharmacy, Hue University, Vietnam.

Chemicals and solvents

The reference standard used was strychnine hydrochloride (P.Code 329824573, Sigma-Aldrich). Methanol (MeOH), acetonitrile (ACN), phosphoric acid (H_3PO_4), triethylamine ($(C_2H_5)_3N$), and double-distilled water of HPLC grade were used as solvents. All chemicals and reagents employed in the study met analytical grade requirements.

Instruments

The analysis was performed using a Shimadzu LC-20AD HPLC system equipped with a photodiode array (PDA) detector (Shimadzu Corporation, Kyoto, Japan). Chromatographic separation was carried out on InertSustain™ C18 and InertSustain™ C8 columns (4.6 × 250 mm, 5 µm) supplied by Shimadzu. Other instruments included an analytical balance (Mettler-Toledo International Inc., Greifensee, Switzerland; readability 0.1 mg), an ultrasonic bath (Elma Schmidbauer GmbH, Singen, Germany), a Z326K centrifuge (Hermle Labortechnik GmbH, Wehingen, Germany), a sens ION™ PH3 pH meter (HACH Company, Colorado, USA), and a Vortex Mixer 250VM (HSi, Seoul, Republic of Korea).

2.2. Methods

Preparation of stock standard solution

An accurately weighed amount of 10.0 mg of strychnine reference standard was transferred into a 10 mL volumetric flask. The solution was made up to volume with MeOH and sonicated to ensure complete dissolution, affording a strychnine stock solution (1000 µg/mL).

Optimization of chromatographic conditions

The proposed chromatographic conditions were initially fixed as follows: injection volume of 5 µL, column oven temperature maintained at 25 *°C (ambient temperature), and detection wavelength of the PDA detector set at 263 nm.* Subsequently, chromatographic conditions were optimized by investigating different stationary phases, including an InertSustain™ C18 column (4.6 × 250 mm; 5 µm) and an InertSustain™ C8 column (4.6 × 250 mm; 5 µm). Various mobile phase systems were evaluated, consisting of MeOH–buffer and ACN–buffer mixtures, where the buffer solution contained 0.3% phosphoric acid (H_3PO_4) and 0.2% triethylamine ($(C_2H_5)_3N$) (buffer PTA). Different solvent ratios and flow rates were examined to select the optimal chromatographic conditions.

Sample preparation procedure

Approximately 200 mg of the powdered herbal sample was accurately weighed and transferred into a 15 mL Falcon tube, followed by the addition of 4.0 mL of MeOH. The mixture was vortex-mixed for 1 min to ensure thorough homogenization. Sample extraction was carried out using ultrasonic-assisted extraction (UAE) at 50 °C for 30 min. After extraction, the mixture was centrifuged at 4000 rpm for 5 min to remove solid impurities. The resulting supernatant was diluted ten-fold with MeOH and subsequently filtered through a 0.45 µm nylon membrane filter. The filtered extract was used for the quantitative determination of strychnine by HPLC. All samples were prepared and analyzed in triplicate to ensure accuracy and reliability. Analytical results were expressed as the mean ± standard deviation (SD) of three independent determinations [6].

Method validation

The analytical method was validated in accordance with the AOAC guidelines [7]. Validation parameters included system suitability, specificity, linearity range, precision, accuracy, limit of detection (LOD), and limit of quantification (LOQ).

3. RESULTS

3.1. Optimization of chromatographic conditions

The chromatographic conditions were systematically investigated to select an appropriate mobile phase system and operating parameters for the separation and quantification of strychnine. The investigation focused on evaluating the effects of the organic solvent type, mobile phase composition, flow rate, and stationary phase on peak characteristics and separation performance of the analyte.

Initially, the chromatographic performance on an InertSustain™ C8 column (250 × 4.6 mm, 5 µm) was evaluated using a MeOH–PTA buffer mobile phase at a low flow rate to assess the retention behavior and peak shape of strychnine. Subsequently, an InertSustain™ C18 column (250 × 4.6 mm, 5 µm) was investigated using mobile phases comprising ACN or MeOH with the same buffer system. Various mobile phase compositions and flow rates (0.5–0.7 mL/min) were examined to optimize retention time, peak symmetry, and resolution. Representative results are presented in Figure 1, supporting the selection of optimal chromatographic conditions.

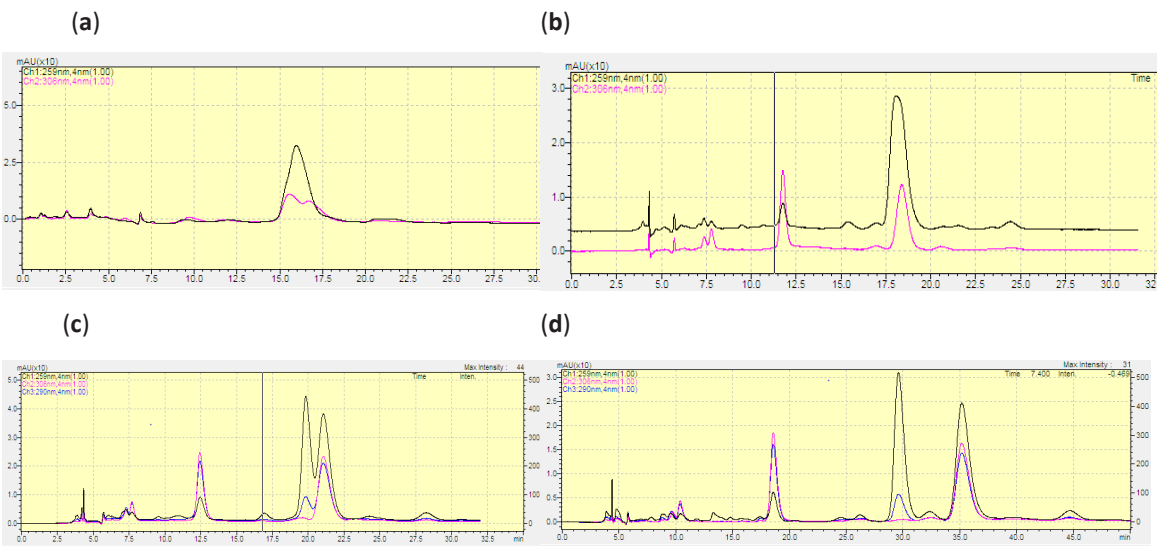


Figure 1. Representative chromatographic conditions employed for the determination of strychnine

Under the initial chromatographic conditions, using MeOH/PTA buffer (30:70, v/v) at a flow rate of 0.45 mL/min on an InertSustain™ C8 column (250 × 4.6 mm, 5 µm), the analyte peaks exhibited poor shape, marked asymmetry, and inadequate resolution, indicating that these conditions were unsuitable for further optimization (Fig. 1a). When the mobile phase was changed to ACN/PTA buffer (15:85, v/v) and the analysis was performed at 0.7 mL/min on an InertSustain™ C18 column, the analytes co-eluted as a single overlapping peak, demonstrating insufficient separation efficiency (Fig. 1b). Lowering the flow rate to 0.6 mL/min under the same mobile phase and column conditions produced a slight improvement in separation; although baseline resolution was still not achieved, the chromatographic behavior suggested that further optimization was feasible (Fig. 1c). Further adjustment of the mobile phase composition to ACN/PTA buffer (10:90, v/v) at 0.5 mL/min resulted in complete resolution and improved peak symmetry; however, the retention time was considerably prolonged (Fig. 1d). The optimal chromatographic performance was ultimately achieved using ACN/PTA buffer (12:88, v/v) at a flow rate of 0.5 mL/min on the InertSustain™ C18 column, which provided complete resolution, satisfactory peak symmetry, and an acceptable retention time, thereby fulfilling the analytical requirements for strychnine determination (Fig. 2d).

3.2. Method validation

System suitability

System suitability was evaluated by injecting the strychnine standard solution six times consecutively. Chromatographic parameters including retention time (t_R), peak area (S), number of theoretical plates (N), tailing factor (T_f), and resolution (R_S) were determined. The results are summarized in Table 1.

Table 1. Results of system suitability testing (n = 6)

Result	t_R (min)	S (mAu.s)	N	T_f	R_S
± SD	32.308 ± 0.52	1724051 ± 18197	4281 ± 77	1.278 ± 0.01	1.591 ± 0.01
RSD (%)	1.61	1.06	1.78		

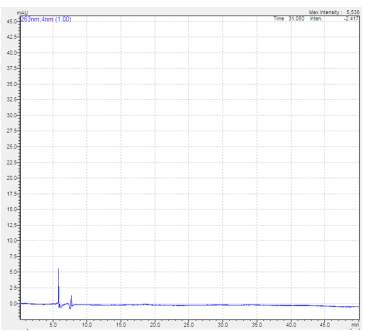
The results presented in Table 1 indicate that the chromatographic system was suitable for the quantitative determination of strychnine in the analyzed samples. Specifically, the relative standard deviations (RSD%) of both the retention time (t_R) and peak area were less than 2%. The number of theoretical plates (N) exceeded 1000, the tailing factor (T_f) was within the acceptable range of 0.8–1.5, and the resolution (R_S) was greater than 1.5.

Selectivity

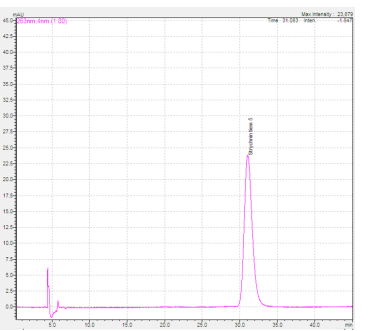
The analyzed samples included a blank sample (MeOH), a strychnine standard solution, a herbal sample, and a spiked herbal sample. The corresponding chromatograms are presented in Figure 2.

The chromatogram of the herbal sample showed a peak at a retention time of 32.308 min, which coincided with the retention time of strychnine in the standard solution (Figure 2b, 2c and 2d). In addition, the UV spectrum of this peak was consistent with that of the strychnine standard (Figure 2e). For the sample solvent (MeOH) (Figure 2a), no strychnine peak was detected, and no interfering or extraneous peaks were observed. These results confirm that the proposed method exhibits high specificity and selectivity for strychnine.

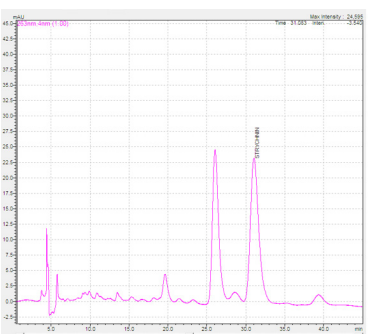
a



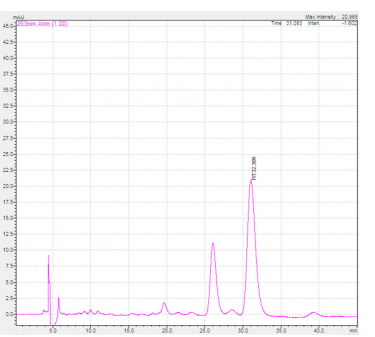
b



c



d



e

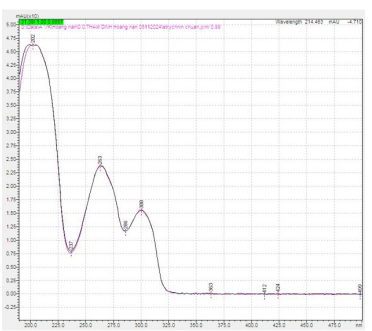


Figure 2. Chromatograms of the samples: MeOH solvent blank (a), strychnine standard solution (b), herbal sample (c), spiked herbal sample (d), and overlay of UV–Vis absorption spectra between the strychnine standard peak and the strychnine peak in the herbal sample (e).

Linearity range

Six strychnine standard solutions with concentrations ranging from 40 to 800 µg/mL were analyzed. The relationship between strychnine concentration and the corresponding peak area was established. The results of this study are presented in Figure 3.

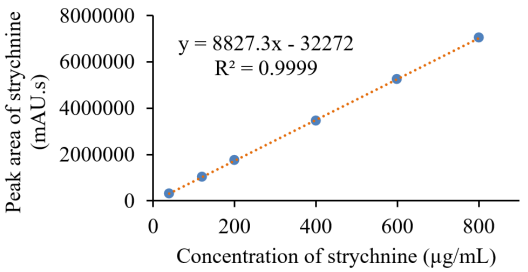


Figure 3. Calibration curve showing the relationship between peak area and strychnine concentration

Within the concentration range of 40–800 µg/mL, the analytical results demonstrated a strong linear correlation between peak area and strychnine concentration, with a correlation coefficient (R^2) of 0.9999.

Precision and accuracy

The precision of the method was evaluated by repeated analysis of six independent herbal samples, quantified under identical analytical conditions on two different days. The analytical results are presented in Table 2.

The accuracy of the method was assessed using the standard addition technique. Briefly, known amounts of strychnine standard solution were spiked into the herbal sample at three concentration levels corresponding to 80%, 100%, and 120% of the endogenous strychnine content (n = 3 for each level). After quantification of the spiked samples, the recovery of strychnine was calculated, and the results are summarized in Table 2.

Table 2. Results of precision and accuracy validation

	Precision (n = 6) (%)		Accuracy (n = 3)		
	Intra-day	**Inter-day**	**+ 80%**	**+ 100%**	**+ 120%**
± SD	3.98 ± 0.02	3.97 ± 0.03	97.93	98.57	96.81
RSD	0.50	0.76	1.01	0.82	1.12

The analytical results demonstrated that the intra-day and inter-day precision of the method exhibited relative standard deviation (RSD) values ranging from 0.50% to 0.76%. Accuracy was assessed using recovery studies at three concentration levels. The obtained recoveries ranged from 96.81% to 98.57%, with RSD values of 0.82%–1.12%. These results comply with AOAC acceptance criteria, which specify adequate precision at RSD < 1.5% and acceptable recovery within the observed range. Therefore, the proposed method demonstrates satisfactory accuracy and precision.

Limit of detection (LOD) and limit of quantification (LOQ)

Diluted solutions prepared from the standard stock solution were analyzed by evaluating the signal-to-noise (S/N) ratio. The LOD and LOQ were determined at S/N ratios of 3 and 10, respectively. The results indicated that the LOD and LOQ of the method were 0.3 µg/mL and 1.0 µg/mL, respectively.

4. DISCUSSION

4.1. Extraction conditions

Among the various extraction techniques evaluated—including ultrasound-assisted extraction, heat reflux extraction, shaking extraction, and cold percolation—ultrasound-assisted extraction demonstrated superior performance. According to Manika N. *et al.*, this method markedly improved the recovery of alkaloids from medicinal materials, achieving extraction efficiencies more than 1.3-fold higher than heat reflux extraction, nearly twofold higher than shaking extraction, and approximately 3.3-fold greater than cold percolation. In addition to enhancing extraction yield, ultrasound-assisted extraction also reduced processing time and energy consumption compared with conventional techniques. Collectively, these findings underscore the significant role of this approach in improving the efficiency of phytochemical extraction and highlight its considerable potential for application in pharmaceutical research and the production of natural product–based therapeutics [8,9,10].

Moreover, previous studies on alkaloid extraction from medicinal plants have reported that the optimal extraction conditions are a temperature of 50 °C and an extraction time of 30 min. This can be attributed to the effective combination of temperature and time, which enhances the release of bioactive compounds from plant matrices. Specifically, a temperature of

50 °C promotes diffusion rates and reduces solvent viscosity, thereby facilitating mass transfer during the extraction process. In the present study, ultrasonic extraction was applied under these conditions (50 °C for 30 min) to optimize the extraction yield of alkaloids from the herbal material. The selection of these parameters complies with conditions validated in previous studies and contributes to improved extraction efficiency, ensuring the quality and reproducibility of the extraction process [6].

4.2. Optimization of chromatographic conditions

During the evaluation of the InertSustain™ C8 column (250 × 4.6 mm, 5 µm), a mobile phase consisting of MeOH and buffer PTA at a ratio of 30:70 (v/v) and a flow rate of 0.45 mL/min was investigated. The results showed relatively short retention times; however, the chromatographic peaks were poorly defined and lacked symmetry. At a detection wavelength of 306 nm, peak overlap was observed at a retention time of approximately 16 min. Despite the use of a low flow rate and a high buffer proportion, satisfactory separation was not achieved. These findings suggest that the InertSustain™ C8 column did not provide sufficient selectivity or separation efficiency, leading to peak co-elution. Consequently, further investigation under alternative chromatographic conditions, including modifications of the stationary phase and mobile phase composition, was required to improve resolution and separation efficiency.

Subsequently, the analytical method was evaluated using a mobile phase composed of ACN and buffer PTA at different mobile phase ratios and flow rates on an InertSustain™ C18 column (250 × 4.6 mm, 5 µm). Preliminary results indicated that at a mobile phase ratio of 15:85 (v/v) and a flow rate of 0.7 mL/min, the retention time was 18.2 min; however, two chromatographic peaks co-eluted into a single peak, indicating incomplete separation and suboptimal elution conditions. Further evaluation at the same mobile phase ratio (15:85, v/v) with a reduced flow rate of 0.6 mL/min resulted in prolonged retention times of approximately 20–21 min. Although complete separation was still not achieved, these results suggest that this mobile phase system exhibits potential, and that improved resolution could be obtained through minor adjustments of chromatographic parameters. Additional optimization experiments were therefore necessary.

When the mobile phase ratio was adjusted to 10:90 (v/v) and the flow rate reduced to 0.5 mL/min, the retention time increased to 35.1 min, and complete separation of chromatographic peaks was achieved. Under these conditions, peak broadening and peak splitting were eliminated, and the peaks exhibited good symmetry and clear resolution. However, the prolonged retention time represents a notable drawback, particularly for applications requiring high analytical throughput. Finally, using a mobile phase ratio of 12:88 (v/v) at a flow rate of 0.5 mL/min, complete peak separation was achieved with appropriate retention times and satisfactory resolution. Notably, the strychnine peak was clearly and completely separated from other components in the sample, indicating high chromatographic resolution. The separation was characterized by sharp, symmetrical peaks without any evidence of overlapping or co-elution, demonstrating that this mobile phase system was the most optimal among all conditions investigated. The retention time of the analyte (~32.3 min) is relatively long; however, this should be considered in relation to the complexity of the *S. wallichiana* matrix. Under the optimized conditions, further reduction of the retention time was not feasible without compromising chromatographic performance, as the achieved resolution (Rs = 1.591) is very close to the minimum acceptable criterion (Rs ≥ 1.5) for baseline separation. Any attempt to shorten the run time resulted in a decrease in resolution and an increased risk of co-elution with matrix components. Despite the extended analysis time, the method remains applicable for routine analysis. In the context of complex herbal matrices, analytical selectivity and reliability are prioritized over speed, and a run time of approximately 32 min is still acceptable for quality control purposes. Moreover, the method demonstrates adequate resolution, supporting its suitability for routine implementation.

To date, studies on the HPLC quantification of strychnine in *S. wallichiana* remain limited. Gu Z.P. et al. reported an HPLC method for the determination of strychnine in *Strychnos* species using a ZY110 YNG-C18 column and a mobile phase consisting of KH_2PO_4 (0.01 M)–MeOH (73:27, v/v), adjusted to pH 2.5 with 10% H_3PO_4, at a flow rate of 1.0 mL/min [4]. In the present study, strychnine was analyzed using an InertSustain™ C18 column (250 × 4.6 mm, 5 µm) with a mobile phase composed of ACN and a buffer PTA. The use of ACN instead of MeOH provides stronger elution strength, lower system backpressure, and improved chromatographic efficiency. Moreover, the addition of triethylamine

effectively suppresses residual silanol interactions on the stationary phase, resulting in enhanced peak symmetry and reduced tailing for the basic alkaloid strychnine.

4.3. Validation of the analytical procedure

The HPLC method developed in this study was validated for system suitability based on key chromatographic parameters, including retention time (t_R), peak area (S), resolution (R_s), number of theoretical plates (N), and tailing factor (T_f). These parameters are fundamental indicators for evaluating the precision, separation efficiency, and stability of a chromatographic system during analysis. The validation results demonstrated that all chromatographic parameters met stringent international requirements, particularly those specified in the AOAC guidelines [7].

Specifically, the relative standard deviations (RSDs) of retention time and peak area were less than 2%, indicating excellent system stability and high precision in peak identification and quantification. The resolution (Rs) values exceeded 1.5, confirming adequate separation between adjacent peaks and the absence of co-elution. The number of theoretical plates (N) was greater than 1000, reflecting high column efficiency and ensuring sharp and well-defined chromatographic peaks. Notably, the tailing factor (Tf) ranged from 0.8 to 1.5, demonstrating effective control of peak tailing and acceptable peak symmetry. Collectively, these results confirm that the HPLC system provides high precision, good resolution, and robust performance, thereby ensuring reliable quantification of strychnine in *S. wallichiana*. Furthermore, the HPLC method exhibited high specificity, as evidenced by the clear agreement in retention time of strychnine and the close similarity of UV spectra among the standard solution, test sample, and spiked sample. These findings confirm the method's ability to accurately distinguish strychnine from other constituents present in the herbal matrix, thereby ensuring analytical specificity.

The linearity of the method was established over a wide concentration range from 40 to 800 µg/mL. This range broadens the applicability of the method for analyzing compounds with varying concentrations in herbal materials and provides sufficient flexibility for routine analysis of samples with different analyte levels. The regression equation demonstrated a strong linear relationship between strychnine concentration and peak area, with a correlation coefficient (R^2) greater than 0.99 across the entire calibration range, indicating high accuracy and reliability of the method. The precision of the method was further confirmed by the low RSD values of the quantified strychnine content, which were within acceptable limits, ensuring consistency and reliable trueness of the analytical results. Overall, the validated HPLC method fulfills the requirements for precision, specificity, and reliability, and can be effectively applied to quality control and quantitative analysis of herbal materials.

4.4. Significance of strychnine analysis in *S. wallichiana*

At present, no studies conducted in Vietnam have reported the quantitative determination of strychnine in the medicinal plant *S. wallichiana*. According to the Vietnamese Pharmacopoeia V, the current assay for *S. wallichiana* is limited to the determination of total alkaloid content, while no specific method is provided for the quantification of the principal active constituent, strychnine [11]. This limitation poses significant challenges to quality control, safety assessment, and standardization of the raw material, particularly given that strychnine is a highly potent alkaloid with a narrow therapeutic window and considerable toxicity, which may lead to poisoning if used without strict dosage control.

From a pharmacological perspective, at very low doses, strychnine was historically used in traditional medicine as a central nervous system stimulant, enhancing neuromuscular activity, alleviating fatigue, reducing pain, and stimulating appetite. However, due to its high toxicity, strychnine is no longer widely employed in clinical practice and is currently permitted only in exceptional cases at extremely low doses under strict medical supervision. Despite its toxic nature, strychnine remains an important compound in pharmacological and neurophysiological research, particularly for elucidating mechanisms related to the activation and regulation of the central nervous system, as well as the physiological responses to inhibitory neurotransmission.

In this context, the development and validation of an accurate and reliable analytical method for the quantification of strychnine in *S. wallichiana* are essential. The results of the present study contribute to the improvement of quality control procedures and the standardization of *S. wallichiana* as a medicinal material. By ensuring that strychnine content is maintained within safe therapeutic limits, the proposed method provides a solid scientific basis for accurate dosage prescription in traditional

medicine, minimizes the risk of clinical toxicity, and establishes a foundation for further research as well as the sustainable utilization and development of this valuable medicinal plant in modern medical practice [12-14].

5. CONCLUSION

This study developed and validated an RP-HPLC method for the quantification of strychnine in *S. wallichiana*. Chromatographic separation was achieved using an acetonitrile–buffer mobile phase (0.3% H_3PO_4 containing 0.2% $(C_2H_5)_3N$) at a ratio of 12:88 (v/v), a flow rate of 0.5 mL/min, and an InertSustain™ C18 column (4.6 × 250 mm; 5 µm), with PDA detection at 263 nm. The proposed RP-HPLC method was validated in accordance with AOAC guidelines and demonstrated acceptable precision, specificity, and repeatability. Therefore, it is suitable for routine quality control and determination of strychnine in *S. wallichiana* herbal materials.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGMENT

This research was funded by University of Medicine and Pharmacy, Hue University (ID No. 18SV/24)

REFERENCES

1. Veera Kishore I, Ragalatha R, Krishna N, Vijayalakshmi M, Mallikarjuna K. Antimicrobial screening of different solvent extracts of an endangered medicinal plant, Strychnos wallichiana Steud ex DC. World Journal of Pharmaceutical Research. 2018;7(3):129-142.

2. Phakeovilay C, Vu NB, Pham TTH, Dang TNL, Do TBD, Nguyen QC, Do Q, Huynh TTN, Nguyen TND, Nguyen P. Determination of strychnine and brucine by HPLC for better quality control of Strychni semen. Journal of Medicinal Materials. 2024;29(6):340-346.

3. Mallikharjuna PB, Seetharam YN, Radhamma MN. Phytochemical and antimicrobial studies of Strychnos wallichiana Steud ex DC. J Phytol. 2010;2(3):22-7.

4. Gu ZP, Ma YJ, Wang JX, Zhang J, Huang YY. Determination of strychnine and brucine in Strychnos by HPLC. Yao Xue Xue Bao. 1997;32(10):791-4.

5. Zhang Z, Liang X, Li P, Zheng L. Capillary electrophoresis with field-enhanced stacking for rapid and sensitive determination of strychnine and brucine. Anal Bioanal Chem. 2003;376(2):210-3.

6. Nguyen KV, Nguyen LKT, Le NT, Ho DV, Heinämäki J, Raal A, Nguyen HT. Sustainable extraction of the tetrahydropalmatine alkaloid from Stephania rotunda Lour. tubers using eco-friendly solvent systems. ACS Omega. 2025;10(35):40313–40323.

7. Official Methods of Analysis of AOAC International: OMA, 22nd Ed.; Latimer, G. W.; Horwitz, W.; AOAC International, Eds.; AOAC International: Oxford New York, NY, 2023.

8. Bhadange YA, Carpenter J, Saharan VK. A Comprehensive Review on Advanced Extraction Techniques for Retrieving Bioactive Components from Natural Sources. ACS Omega. 2024;9(29):31274–31297.

9. Manika N, Gupta VK, Verma RK, Darokar MP, Pandey N, Bagchi GD. Extraction efficacy, antibacterial potential and validation of RP-HPLC coupled with diode array detection in Holarrhena pubescens. International Journal of Pharmaceutical Sciences and Research. 2013;4(8):3020-3027.

10. Nguyen VK, Nguyen TKL, Ngo TB, Hoang CTN, Tran HH, Cao TH. Development of a quantitative method for conessine and in vitroevaluation of the cytotoxic and anti-inflammatory activities of Holarrhena pubescens (Buch.-Ham.) Wall. ex G. Don. Hue Journal of Medicine and Pharmacy. 2025;15(3):126–133.

11. Ministry of Health. Vietnamese Pharmacopoeia V. Medical Publishing House; 2017. p. 1192.

12. Xu L, Zhang Y, Bian Y, Zhou Y, Feng X song. Progress of Pretreatment and Analysis Methods for Brucine and Strychnine: An Update Since 2013. Critical Reviews in Analytical Chemistry. 2025 Mar 28;0(0):1–20.

13. Zlotos DP, Mandour YM, Jensen AA. Strychnine and its mono- and dimeric analogues: a pharmaco-chemical perspective. Nat Prod Rep. 2022 Oct 19;39(10):1910–37.

14. Chen J, Qu Y, Wang D, Peng P, Cai H, Gao Y, et al. Pharmacological Evaluation of Total Alkaloids from Nux Vomica: Effect of Reducing Strychnine Contents. Molecules. 2014 Apr;19(4):4395–408.