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Quality and safety evaluation of *Andrographis* products using a USP-modified HPLC-PDA single-standard approach

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ABSTRACT: *Andrographis paniculata* (Burm.f.) Wall. ex Nees is widely used in traditional medicine, particularly in Asia, due to its immunomodulatory, anti-inflammatory, and antiviral properties. Andrographolide (AD) and 14-deoxy-11,12-didehydroandrographolide (14-DAD) are key diterpene lactones commonly used as markers for quality and safety evaluation. This study aimed to evaluate the quality and safety of fifteen commercially available *Andrographis* products using a validated HPLC-PDA method modified from the USP 43–NF 38 monograph. All parameters, including specificity, linearity and range, accuracy, precision, and the limit of quantitation, met all requirements in accordance with ICH Q2(R2) guidelines. Quantitative analysis showed that AD was present in all samples, ranging from 0.38% to 5.08% (w/w), of which two products failed to comply with pharmacopeial specifications. Based on their recommended daily dose, six products contained insufficient amounts of their active ingredients to achieve the therapeutic effect. Notably, 14-DAD, a vasodilator, was detected in all samples. Among these, eleven products exhibited high levels of 14-DAD, with eight samples (53.3%) exceeding the USP 43–NF 38 limit and three reaching borderline levels. These findings raise significant safety concerns, particularly regarding cardiovascular risk associated with 14-DAD exposure. In conclusion, many products do not conform to the established quality and safety standards. The validated HPLC-PDA method developed in this study provides a reliable and practical approach for the routine quality control and standardization of *Andrographis* products.

KEYWORDS: *Andrographis paniculata*, andrographolide, 14-deoxy-11,12-didehydroandrographolide, diterpene lactones, product quality and safety, method validation, HPLC-PDA

INTRODUCTION

Andrographis paniculata (Burm.f.) Wall. ex Nees, commonly known as "Fa Thalai Chon" in Thailand, is a medicinal plant widely used in traditional Asian medicine for the treatment of fever, sore throat, respiratory infections, diarrhea, and inflammatory conditions [1–3]. In recent years, its antiviral activity has gained increasing attention, particularly in the context of the COVID-19 pandemic [4]. The pharmacological activities of *A. paniculata* are primarily attributed to its diterpene lactones, including andrographolide (AD), 14-deoxy-11,12-didehydroandrographolide (14-DAD), neoandrographolide (NAD), and andrograpanin (ADG) (Fig. 1). Among these constituents, AD is the predominant compound and is widely recognized as a marker for quality control. In contrast, 14-DAD has been reported to exhibit vasorelaxant activity, which may raise safety concerns due to its vasodilatory effects, particularly in individuals with underlying cardiovascular conditions [5, 6].

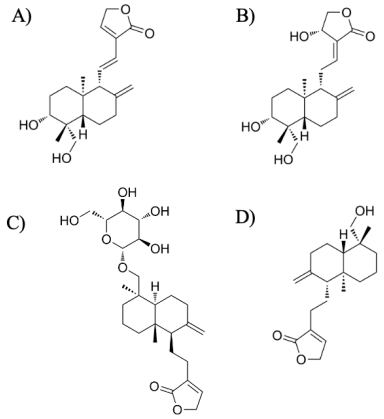

Fig. 1 Chemical structures of four diterpene lactones in *A. paniculata*: (A) 14-deoxy-11,12-didehydroandrographolide (14-DAD), (B) andrographolide (AD), (C) neoandrographolide (NAD), and (D) andrograpanin (ADG).

Pharmacopeial standards, such as those established by the United States Pharmacopeia 43–National Formulary 38 (USP 43–NF 38), define acceptable limits for key bioactive constituents in *Andrographis* products. Specifically, the content of AD must be not less than 1% (w/w), while 14-DAD must not exceed 15% of the total diterpene lactones. However, in several Asian countries, regulatory specifications for 14-DAD remain undefined [7]. In Thailand, the Thai Herbal Pharmacopoeia 2021 (THP 2021) specifies only the minimum content of AD and does not establish limits for 14-DAD, thereby creating a potential gap in safety regulation. This context emphasizes the importance of

comprehensive quality assessment to ensure both the safety and efficacy of commercial products.

High-performance liquid chromatography (HPLC) is a well-established analytical technique for the quantification of bioactive compounds in herbal products, offering high sensitivity and specificity, thereby enabling accurate determination of active constituents [8]. Conventional methods typically require individual reference standards for each compound, which may limit their routine applicability. The USP addresses this constraint by proposing a single-standard approach using AD in combination with relative response factors, also referred to as conversion factors, for the quantification of related diterpene lactones. In USP 43–NF 38, an HPLC method coupled with photodiode array detection (HPLC-PDA) is recommended as the compendial method for the analysis of diterpene lactones in *Andrographis* products [9].

Therefore, this study aimed to evaluate the quality and safety of commercially available *Andrographis* products in Thailand using a validated HPLC-PDA method adapted from USP 43–NF 38, employing AD as a single reference standard for the quantification of four diterpene lactones. In addition, this study provides a validated approach for assessing 14-DAD content using this method.

MATERIALS AND METHODS

Chemicals and reagents

Andrographolide DMSc (98.45%; control No. 01B63213) was purchased from the Department of Medical Sciences, Ministry of Public Health, Thailand. Authentic reference standards: AD, NAD, 14-DAD, and ADG, for confirmation of the predicted relative relation time (RRT) values were obtained from NSTDA Characterization and Testing Service Center, National Science and Technology Development Agency, Thailand. *A. paniculata* leaves powdered was acquired from herbal drugstores in Bangkok, Thailand, and compared with an authentic powdered plant sample as specified in the Thai Herbal Pharmacopoeia 2021 [10]. Fifteen legally registered *Andrographis* products, each within one year of the manufacturing date, were purchased from local drugstores in Thailand.

Methanol and acetonitrile, HPLC grade, were provided by Fischer Science (South Korea). All other chemicals were of analytical grade. Ultra-pure water was obtained using a Simplicity® water purification system (Merck, USA).

Instrumentation

Chromatographic analysis was conducted on a Shimadzu® LC-20 HPLC system, which included a system controller (CBM-20A), solvent delivery pump (L-20AD), degasser unit (DGU-20A 5R), automatic injector (SIL-20AC HT), and diode array detector (SPD-M20A). Data collection and processing were carried out using LC Solution® software.

Chromatographic condition

A Hypersil BDS C18 column (250 × 4.6 mm, 5 µm; Thermo Scientific™, USA) was used as the stationary phase. The mobile phase consisted of two solutions: solution A, a potassium dihydrogen phosphate buffer (0.14 g/l) with 0.5 ml phosphoric acid, and solution B, acetonitrile. Gradient elution was performed over a 45-min time course as follows: 0–18 min, 5–45% B; 18–25 min, 45–80% B; 25–28 min, 80% B; 28–35 min, 80–45% B; 35.0–40.0 min, 45–5% B; holding at 5% B for the final 5 min. The flow rate was set to 1.5 ml/min. The injection volume was 20 µl, and UV detection was at 223 nm.

Preparation of standard solution

An accurately weighed andrographolide (DMSc standard) was dissolved in methanol to achieve a concentration of 1.0 mg/ml. A 5.0 ml of this solution was transferred into a 10 ml volumetric flask, diluted to volume with acetonitrile, and mixed thoroughly.

Preparation of sample solution

Two grams of fine powdered leaves (No. 180 mesh sieve) were accurately weighed into a 100 ml volumetric flask. Seventy milliliters of 50% methanol was added, and the mixture was then sonicated at room temperature for 15 min. The solution was adjusted to volume with 50% methanol [11].

Identification of Relative Retention Time in *A. paniculata* analysis

To verify the accuracy of RRT, a comparative study was conducted across two laboratories in accordance with the *Andrographis* monograph (USP 43–NF 38).

Laboratory 1 (Lab 1): An *A. paniculata* solution was prepared to evaluate chromatographic separation efficiency. Four diterpene lactones were characterized based on their RRTs, calculated as the ratio of each analyte's retention time (RT) to that of andrographolide (AD).

Laboratory 2 (Lab 2): Individual 125 mg/ml solutions of four authentic reference standards were first analyzed to determine their specific RTs. This was followed by the analysis of a mixed standard and an *A. paniculata* powder solution to assess system efficiency.

Constituents in the sample were identified by comparing them with the mixed standard chromatogram. RRTs in the sample solution were then calculated relative to the RT of AD.

Method validation

System suitability

The system suitability of the analytical method was evaluated by assessing peak symmetry, tailing factor,

retention time, theoretical plate number, and resolution. Chromatograms of the standard and sample solutions were examined. The RT of AD in the sample corresponded to that of the standard. Each diterpene lactone was identified based on its RRT, calculated as the ratio of its retention time to that of AD [9].

Specificity

The specificity of the analytical method was evaluated by comparing the chromatograms of the test sample, the AD standard, and the blank. Each analyte peak showed no interference from other peaks.

Linearity and range

Linearity and range were evaluated in accordance with the ICH Q2(R2) guideline using five concentration levels of finely powdered leaf samples (12, 16, 20, 24, and 30 mg/ml), each analyzed in five replicate injections [12]. The contents of AD, NAD, 14-DAD, and ADG in the powdered samples were determined using the equation specified in the *Andrographis* monograph (USP 43–NF 38) [9]. Calibration curves were constructed by plotting peak area versus concentration, and linear regression analysis was performed. The coefficient of determination (r^2) for all analytes was greater than 0.995, indicating excellent linearity in accordance with AOAC 2016 criteria [13].

Precision

The precision for each compound was evaluated by determining repeatability (intra-day) and intermediate precision (inter-day). For repeatability, three concentrations of leaf powder (12, 20, and 30 mg/ml) were injected in five replicates within the same day. Intermediate precision was determined by analyzing the same concentrations with 5 replicates each over three different days. Precision was assessed by calculating the percentage of relative standard deviation (%RSD) of each testing [13].

Accuracy

The method accuracy was evaluated by determining the percentage recovery (%R), calculated by comparing the measured concentrations of each compound in the samples with the corresponding theoretical values derived from the regression equation [13]. Three concentration levels (12, 20, and 30 mg/ml) were analyzed, each with five replicate injections. The contents of the four compounds at each concentration level were calculated using the equation specified in the *Andrographis* monograph, as described in Eq. (1) [9].

Limit of quantification (LOQ)

The LOQ was defined as the lowest concentration that can be quantified with acceptable accuracy and precision. This concentration was evaluated in ten replicates, then precision and accuracy were determined. Based on three linearity curves, LOQ was calculated using the equation [12];

$$\mathrm{LOQ} = 10 \times (\sigma / S)$$

where σ is the standard deviation of the response and S is the slope of the calibration curve.

Determination of diterpene lactones in *Andrographis* products

Twenty units of the *Andrographis* product were weighed to calculate the average weight of each. For each sample, an accurate portion of 2.0 g of the homogenized fine powder was transferred to a 100 ml volumetric flask, and 70 ml of 50% methanol was added. The mixture was sonicated at room temperature for 15 min, and then the volume was adjusted. The extracted solution was diluted to the suitable final concentration. All sample solutions were filtered through a 0.45 µm membrane filter before injection. The percentage content of each compound in the sample portion was calculated using the following equation, incorporating a conversion factor, based on USP 43–NF 38 [9]. The total diterpene lactone content was calculated as the sum of AD, 14-DAD, NAD, and ADG.

$$\text{Percentage content of each compound (\%)} = (A_u/A_s) \times (C_s/W) \times 10 \times F \quad (1)$$

where A_u is the peak area of each identified diterpene lactone in the sample solution, A_s is the peak area of AD in the AD (DMSc) standard solution, C_s is the concentration of AD (DMSc) in the standard solution, W is the weight of the sample taken for analysis, F is the conversion factor (1.00 for AD, 3.90 for NAD, 1.45 for 14-DAD, and 2.65 for ADG).

Statistical analysis

Data were analyzed in five replicates and are presented as the mean ± standard deviation (SD). Statistical significance ($p < 0.05$) was determined by one-way ANOVA followed by Dunnett's t-test for multiple comparisons.

RESULTS AND DISCUSSION

Several studies have established HPLC-based methods for the quality control of *A. paniculata*. For instance, Pholphana et al developed an isocratic HPLC method using a methanol–water mobile phase at 220 nm for the simultaneous determination of three diterpene lactones (AD, 14-DAD, and NAD) using in-house purified standards [14]. Similarly, Karioti et al [15] used an HPLC-DAD–MS analysis to identify constituents, including andrographaside, andropanoside, AD, NAD, 14-deoxyandrographolide, 14-DAD, and ADG, comparing RT and spectral data with both Chinese Pharmacopoeia and in-house isolated standards. Furthermore, Villedieu-Percheron et al [16] reported a rapid gradient

HPLC-UV method for the quantification of three diterpene lactones (AD, 14-DAD, and NAD) using external standards purified via silica gel flash chromatography. Nonetheless, conventional HPLC methods typically require pure reference standards for each analyte, which are a limitation for routine application in many laboratories.

As reported in previous studies, the analysis of related diterpene lactone compounds in *A. paniculata* requires the reference standards for accurate peak identification of RT. Those reference substances are often costly and not readily available. Consequently, the RRT approach has been widely adopted in pharmacopoeias, and the literature to characterize chromatographic behavior when reference standards are unavailable. In this study, a validated HPLC method employing a single reference standard was developed, based on the *Andrographis* monograph in USP43–NF 38 [9]. This approach significantly reduces analytical cost and enhances feasibility for routine quality control, particularly in resource-limited settings. AD was used as the reference standard for the simultaneous quantification of four major diterpene lactones in the samples including AD, NAD, 14-DAD, and ADG. Each compound was separated using a gradient elution profile and detected by UV at 223 nm. The AD peak of the sample was identified by comparison with the reference standard, and the remaining compounds were characterized based on their RRT, calculated as the ratio of each analyte RT to that of AD obtained in sample.

Due to variability of RRT across different laboratories, discrepancies between experimental and expected values may occur. To address this, a comparative study of two independent laboratories was conducted to evaluate RT and RRT values. The RRT values obtained from Laboratory 1 (Fig. 2A) were consistent with those from Laboratory 2, and both complied with the *Andrographis* monograph (USP 43–NF 38) (Table 1). The RTs of compounds in the sample solution corresponded to those in the mixed standard solution (Fig. 2B), confirming the reliability of RT identification and demonstrating that the method provides consistent RRT values for each diterpene lactone. Method accuracy was further verified through the spike method, with percentage of recovery ranging from 97.9 to 104.2% (Table S1), indicating that this chromatographic condition gives the accurate quantification. Despite the limited availability standard of 14-DAD, NAD, and ADG, the application of analytical protocols in accordance with USP 43–NF 38, a recognized official pharmacopeia, was performed.

Method validation

System suitability was confirmed by analyzing both the AD reference standard and the sample solutions containing the four diterpene lactones. The method exhibited excellent column performance, with a theoretical plate count of 115,738 and a tailing factor of less than 1.5, as calculated using LC Solution™ software in accordance with the USP General Chapter <621> on Chromatography. All analyte peaks were completely separated (resolution > 2.0). Furthermore, the calculated RRT complied with the USP 43–NF 38 criteria (Fig. 2A). These results demonstrate that the modified HPLC-PDA method, employing a gradient elution, is suitable for the quantification of diterpene lactones in *A. paniculata*.

Method specificity was confirmed by comparing the chromatograms of the blank, AD reference standard, and sample solution. No interfering peaks were observed at the RT of the four diterpene lactones in the chromatograms, demonstrating the specificity of the developed analytical method (Fig. 3).

Linearity and range were assessed using five concentration levels of powdered leaf samples, each analyzed in five replicates. Quantification of the four diterpene lactones was performed using a single-standard approach with conversion factors, as described for the determination of diterpene lactones in *Andrographis* products. All analytes exhibited excellent linearity, with coefficients of determination (r^2) greater than 0.999. The linear ranges were 130–301 µg/ml for AD ($r^2 = 0.9995$), 51–119 µg/ml for NAD ($r^2 = 0.9997$), 120–218 µg/ml for 14-DAD ($r^2 = 0.9995$), 16–37 µg/ml for ADG ($r^2 = 0.9992$), and 316–738 µg/ml for total diterpene lactones ($r^2 = 0.9995$) (Table 1).

The developed method demonstrated excellent precision, with both repeatability (intra-day) and intermediate precision (inter-day) yielding %RSD values of less than 2% in all concentration levels (Table 1). The percentage recovery of four diterpene lactones ranged from 97.9% to 104.2%, and the LOQ showed acceptable precision and recovery in accordance with the AOAC 2016 criteria [13].

Overall, these results demonstrate that the modified HPLC-PDA method, utilizing a single reference standard and gradient elution, is suitable for the simultaneous quantification of AD, NAD, 14-DAD, ADG, and total diterpene lactones in *A. paniculata*, supporting its application in routine quality control.

Quantification and evaluation of *Andrographis* products

The quality of *Andrographis* products was evaluated in compliance with both USP 43–NF 38 and THP 2021, which require the specification of various active markers, including AD, 14-DAD, diterpene lactones, and total lactones [9, 17]. In this study, the compounds were identified based on their RRT, and their amounts were calculated using a conversion factor, as previously described.

Fifteen registered *Andrographis* products were purchased from the local market to assess the content of four diterpene lactones: AD, NAD, 14-DAD, and

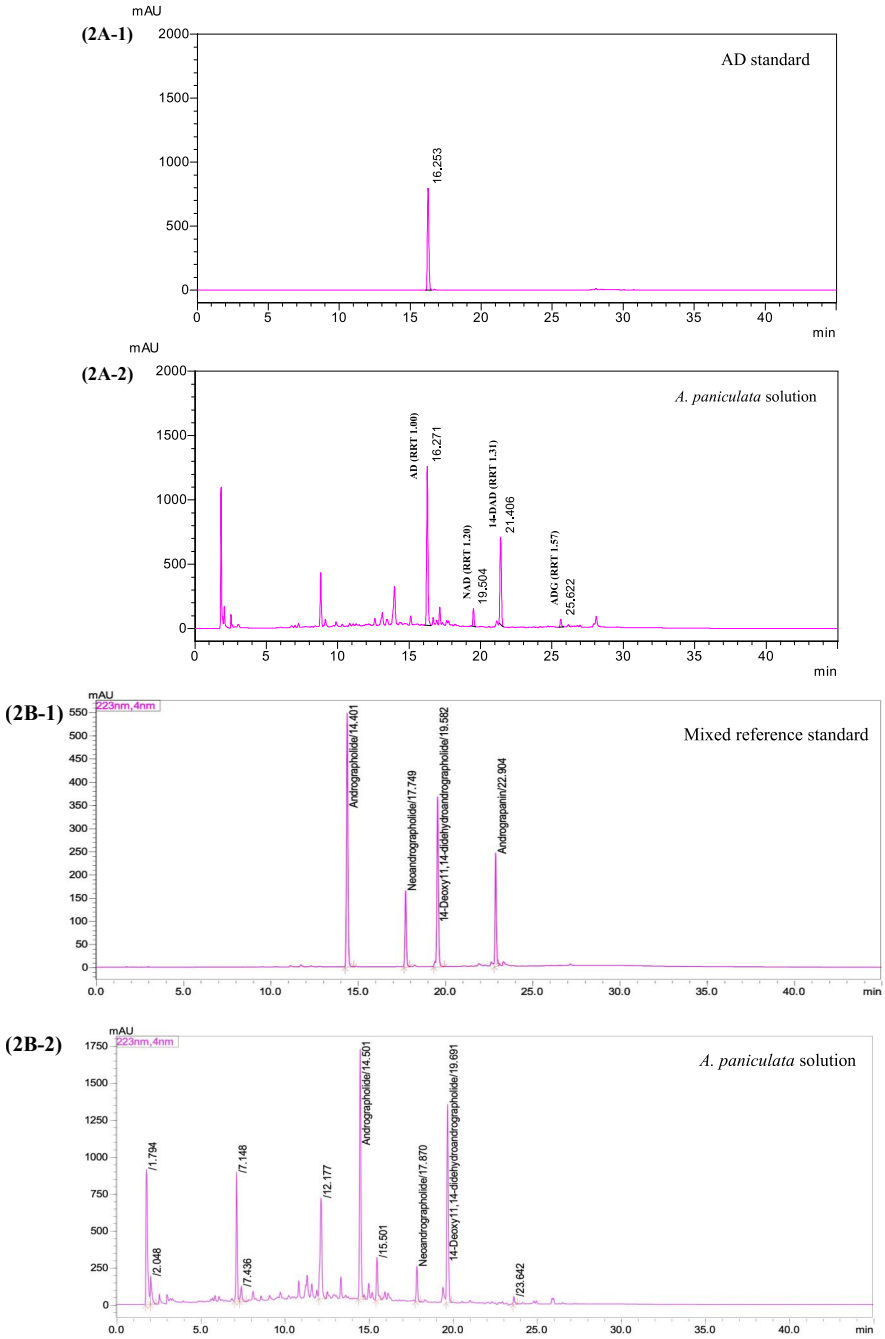

Fig. 2 HPLC chromatograms obtained from two laboratories. 2A (Lab 1): (2A-1) AD reference standard; (2A-2) *A. paniculata* sample solution. 2B (Lab 2): (2B-1) mixed reference standard; (2B-2) *A. paniculata* sample solution.

ADG. The samples were analyzed using a validated HPLC-PDA method in gradient elution mode, employing a single-reference standard approach. Among the fifteen products, five were labeled as leaf powder, four as aerial part powder, three as extract. Additionally, two products were labeled as a combination of aerial part and extract, while one product contained a mixture of *A. paniculata* aerial part and other medicinal plants. Regarding label claims, six of the fifteen products specified their active constituent content: five declared only the percentage of AD, and one declared both AD and total lactones. The remaining nine products did not declare the content of any active constituents. A limitation of this study is the small sample size, as only one batch from each brand was analyzed.

Table 1 Method validation parameters for four compounds and diterpene lactone (the sum of AD, NAD, 14-DAD, and ADG).

Parameter	RT (RRT)	Range	Regression equation	r^2	LOQ (µg/ml)	Precision (%RSD)		Accuracy
	min	(µg/ml)			(Test. LOD ± %RSD; %R)	Repeatability (Intra-day)	Reproducibility (Inter-day)	(% Recovery)
AD	16.27 (1.00)	130–301	y = 27661x–155597	0.9995	17.6 (23.0 ± 0.10; 99.4%)	0.03–0.5	1.8–2.7	96.7–100.2
NAD	19.50 (1.20)	51–119	y = 7080.5x–2594	0.9997	20.6 (20.0 ± 0.80; 98.8%)	0.2–0.5	1.5–4.8	101.2–101.7
14-DAD	21.40 (1.31)	120–281	y = 19056x–83383	0.9995	13.4 (15.0 ± 0.07; 103.2%)	0.06–0.4	0.4–3.1	98.3–100.4
ADG	25.62 (1.57)	16–37	y = 10948x–17826	0.9992	7.2 (8.0 ± 0.57; 104.5%)	0.06–0.7	1.2–2.8	95.6–102.4
Diterpene lactone (AD, NAD,14-DAD, ADG)		316–738	y = 20228x–259400	0.9995	32.6 (51.0 ± 0.07; 101.8%)	0.3–0.7	0.8–2.8	99.8–101.3

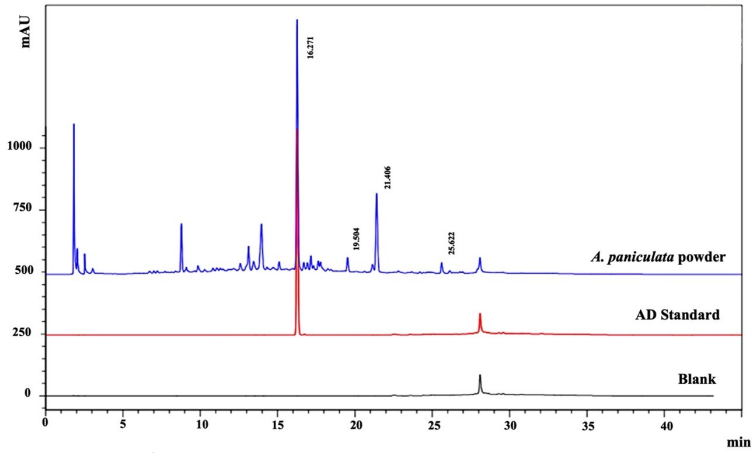

Fig. 3 Overlay HPLC chromatograms of *A. paniculata* powder, AD standard, and blank solution.

The analysis of fifteen commercial products revealed variability in both the composition and content of four diterpene lactones (Fig. 4), which may contribute to inconsistent clinical outcomes among patients using commercially available products. AD and 14-DAD were detected in all products, whereas NAD and ADG were present at lower level and were undetectable in some samples. AD was the predominant

Table 2 Content of compounds (% w/w) in *Andrographis* products.

Product	Preparation	% Content of compounds (w/w), Mean ± SD				
		AD	NAD	14-DAD	ADG	Total diterpene lactones (AD, NAD,14-DAD, ADG)
No. 1	Leaf powder	1.54 ± 0.001	0.33 ± 0.01	0.59 ± 0.01	0.19 ± 0.003	2.65 ± 0.02
No. 2	Aerial powder	1.06 ± 0.03	0.24 ± 0.01	0.47 ± 0.01	0.02 ± 0.003	1.80 ± 0.02
No. 3	Aerial powder	0.71 ± 0.01	0.14 ± 0.002	0.41 ± 0.01	0.06 ± 0.002	1.33 ± 0.01
No. 4	Aerial powder	2.06 ± 0.03	0.23 ± 0.002	0.24 ± 0.01	0.12 ± 0.01	2.65 ± 0.05
No. 5	Aerial powder + Other plants	1.14 ± 0.003	0.18 ± 0.001	0.62 ± 0.01	0.03 ± 0.001	1.97 ± 0.02
No. 6	Leaf powder	2.64 ± 0.01	0.21 ± 0.002	0.84 ± 0.004	0.19 ± 0.001	3.89 ± 0.02
No. 7	Aerial powder + Extract	0.38 ± 0.01	0.06 ± 0.001	0.15 ± 0.004	0.008 ± 0.004	0.66 ± 0.01
No. 8	Aerial powder	1.53 ± 0.001	0.28 ± 0.002	0.44 ± 0.01	0.16 ± 0.001	2.41 ± 0.01
No. 9	Leaf powder	2.36 ± 0.02	0.46 ± 0.01	1.13 ± 0.02	0.27 ± 0.003	4.22 ± 0.01
No. 10	Leaf powder	2.43 ± 0.02	0.30 ± 0.001	0.44 ± 0.01	0.12 ± 0.004	3.29 ± 0.01
No. 11	Extract	4.63 ± 0.21	0.42 ± 0.02	0.88 ± 0.08	0.16 ± 0.01	6.08 ± 0.32
No. 12	Extract	5.08 ± 0.80	0.33 ± 0.004	0.50 ± 0.04	ND	5.91 ± 0.84
No. 13	Extract	2.22 ± 0.06	0.28 ± 0.001	0.43 ± 0.01	ND	2.94 ± 0.07
No. 14	Leaf powder	2.17 ± 0.17	0.25 ± 0.02	0.42 ± 0.03	0.12 ± 0.01	2.96 ± 0.23
No. 15	Aerial powder + Extract	1.85 ± 0.05	ND	0.05 ± 0.01	ND	1.90 ± 0.05

ND = Not detected.

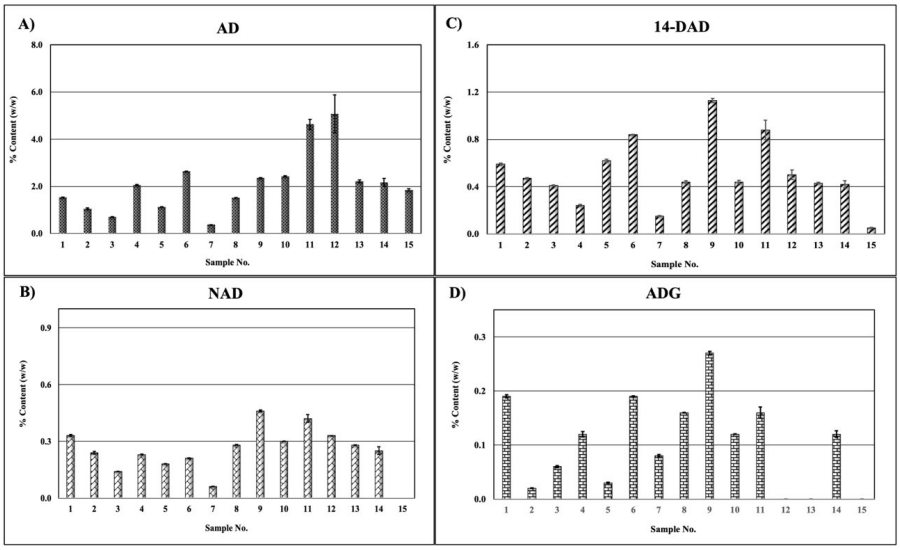

Fig. 4 Bar chart showing the variation in the amounts of four diterpene lactones among *Andrographis* products.

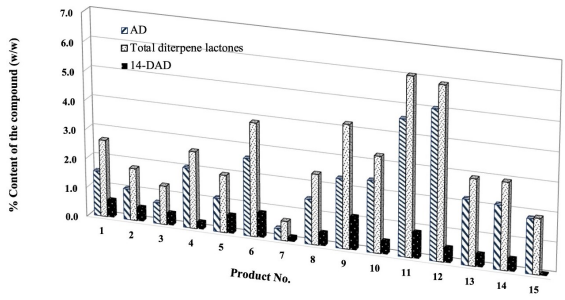

Fig. 5 Comparison of the contents of AD, 14-DAD, and total diterpene lactones within each product.

constituent, with contents ranging from 0.38 ± 0.01% to 5.08 ± 0.80% w/w. The content of 14-DAD ranged from 0.05 ± 0.01% to 1.13 ± 0.01% w/w, while NAD levels ranged from non-detectable to 0.46 ± 0.01% w/w. ADG was detected only at trace levels, with a maximum of 0.27 ± 0.003% w/w. Overall, most products contained total diterpene lactones (the sum of AD, 14-DAD, NAD, and ADG) more than 1% (w/w) (Table 2).

Regarding specification standards, the Thai Herbal Pharmacopoeia 2021 (THP 2021) specifies limits for AD content (measured by HPLC/UV) and total lactone content (determined by titration), whereas USP 43–NF 38 specifies the simultaneous determination of AD, 14-DAD, and total diterpene lactones [9, 17]. Based on the criteria, two products (13.3%) failed to meet the AD requirement: Product No. 3, which consisted of aerial part powder, failed to meet the AD specification, while Product No. 7, which contained a combination of aerial part and extract, failed to meet both the AD and total diterpene lactone requirements (Table 2, Fig. 4).

As the second most abundant compound, 14-DAD exhibits vasorelaxant activity and has been utilized as an antihypertensive agent [5, 6]. However, due to safety concerns regarding its cardiovascular effects, USP43–NF 38 restricts 14-DAD content to a maximum of 15 % of the total diterpene lactones (the sum of AD, NAD, 14-DAD, and ADG) [9]. Our study revealed that eight out of fifteen products (53.3%) exceeded this limit. Furthermore, three (No. 11, No. 13, and No. 14) were identified as borderline cases, with 14-DAD ratios nearing the USP maximum limit (Table 3, Fig. 5). Among the non-compliant samples, four (No. 2, No. 3, No. 5, and No.8) were prepared from aerial part powder, three (No. 1, No. 6, and No. 9) from leaf powder, and one (No. 7) from a mixture of powder and extract. NAD and ADG also contribute to the pharmacological profile of the herb, including antipyretic and anti-inflammatory effects, with NAD exhibiting potent antipyretic activity [18]. The amount of NAD was observed high in extract and leaf powder products (No. 9–13), whereas ADG was undetectable in some extract preparations. These findings suggest that although extraction processes may enrich certain active constituents, higher content does not necessarily indicate standardized quality.

According to official therapeutic guideline, the Thai National List of Essential Herbal Medicines recommends a daily AD dose of 60–120 mg for treating common cold symptoms [19]. Furthermore, the Thai Ministry of Public Health recommends a higher daily intake of 180 mg of AD for the treatment of COVID-19 [20]. This study showed significant variations in unit weights and dosage instructions across the samples. Most products specified 300–400 mg per unit; labeling suggested daily intakes ranged widely from four to sixteen units. Although manufacturers specified

Table 3 Comparison of labeled and measured contents of four compounds in *Andrographis* products and evaluation against Pharmacopoeia Standards. * $p < 0.05$.

Product	Dosage form	Preparation	Label weight (mg/unit)	Average weight (mg)	Label amount of active marker constituents	Dose claim (serving/day)	Measured AD				Measured total diterpene lactones (AD, 14-DAD, NAD, ADG) % found (w/w)	Measured 14-DAD % found (w/w)	Calculated 14-DAD in total diterpene lactones % found (w/w)	Evaluation		
							% found (w/w)	per unit (mg)	%LA	per serving size (mg/day)				AD (USP, THP)	Diterpene lactone (USP)	Limited 14-DAD (USP)
No. 1	Capsule	Leaf powder	350	402.7		4 q.i.d.	1.5	6.2		99.2	2.6	0.6	22.3*	Pass	Pass	Fail
No. 2	Capsule	Aerial powder	500	400.0		2-4 q.i.d.	1.1	4.2		67.2	1.8	0.5	26.1*	Pass	Pass	Fail
No. 3	Capsule	Aerial powder	350	257.1		4-5 b.i.d.	0.7	1.8		18.0	1.3	0.4	30.8*	Fail	Pass	Fail
No. 4	Capsule	Aerial powder	390	300.9		2-3 t.i.d.	2.1	6.2		55.8	2.6	0.2	9.1	Pass	Pass	Pass
No. 5	Capsule	Aerial powder + Other plants	400	336.5		1-2 q.i.d.	1.1	3.9		31.2	2.0	0.6	31.4*	Pass	Pass	Fail
No. 6	Capsule	Leaf powder	400	427.3		2-3 q.i.d.	2.6	11.3		135.6	3.9	0.8	21.6*	Pass	Pass	Fail
No. 7	Tablet	Aerial powder + Extract	250	267.9		3 t.i.d.	0.4	1.0		9.0	0.7	0.2	22.6*	Fail	Fail	Fail
No. 8	Tablet	Aerial powder	370	385.0		4 t.i.d.	1.5	5.9		70.8	2.4	0.4	18.2*	Pass	Pass	Fail
No. 9	Capsule	Leaf powder	350	375.1	AD $\not<$ 7 mg/cap	3-4 q.i.d.	2.4	8.9	127.1	142.4	4.2	1.1	26.8*	Pass	Pass	Fail
No. 10	Capsule	Leaf powder	350	352.9	AD $\not<$ 7 mg/cap	3-4 q.i.d.	2.4	8.6	122.9	137.6	3.3	0.4	13.4	Pass	Pass	Pass
No. 11	Capsule	Extract	NA	429.2	AD $\not<$ 20 mg/cap	1 q.i.d.	4.6	19.9	99.5	79.6	6.1	0.9	14.5	Pass	Pass	Pass
No. 12	Capsule	Extract	300	292.3	AD $\not<$ 6 mg/cap	3 t.i.d.	5.1	14.9	248.3	134.1	5.9	0.5	8.5	Pass	Pass	Pass
No. 13	Capsule	Extract	400	433.5	AD 10 mg/cap	1 q.i.d.	2.2	9.6	96.0	38.4	2.9	0.4	14.6	Pass	Pass	Pass
No. 14	Capsule	Leaf powder	350	278.4	AD $\not<$ 1.7 % w/w, Total lactone $\not<$ 6 % w/w	3 t.i.d.	2.2	6.0	127.7	54.0	3.0	0.4	14.2	Pass	Pass	Pass
No. 15	Tablet	Aerial powder + Extract	250	258.2		3 t.i.d.	1.9	4.8	103.3	43.2	1.9	0.1	2.6	Pass	Pass	Pass

b.i.d. = twice a day, t.i.d. = three times a day, q.i.d. = four times a day.

dosages per serving on their labels, this study revealed that six products contained low amounts of AD. Based on therapeutic requirements for treating the common cold, these levels were considered subtherapeutic, with daily doses ranging from 9.0 to 55.8 mg/day (Table 3). Notably, Product No.13 complied with the USP 43–NF 38 for AD, 14-DAD, and diterpene content; however, the actual amount per serving size, calculated from the labeled dose claim, was found below the recommended daily intake (AD, 60–120 mg/day for common cold symptoms). Increasing the dose to achieve the therapeutic AD targets would consequently raise the intake of 14-DAD, potentially increasing safety risks. These findings highlight the importance of the simultaneous quantification of AD and 14-DAD to ensure that dosage regimens remain both effective and safe. Moreover, these data may support individualized therapeutic decision-making by enabling dose adjustments tailored to patient-specific needs.

In addition, *A. paniculata* contains various diterpene lactones, such as 14-deoxyandrographolide, isoandrographolide, 14-deoxy-14, 15-didehydroandrographolide, and others, as well as various flavonoids including 5-hydroxy-7, 8-dimethoxyflavone and 7-O-methylwogonin [21–25]. These compounds may act synergistically to contribute to the herb's therapeutic effects. Extraction processes intended for AD enrichment may reduce the levels of other bioactive compounds which involved in treating fever, sore throat, diarrhoea, and respiratory symptoms including COVID-19 [19, 20]. Notably, AD is sensitive to high temperatures and humidity and degrades into 14-DAD, with a reported half-life of approximately 4.2 years in dried plant powder [17, 26].

Taken together, the results demonstrate that product registration status, manufacturing date, expiration date, and retail distribution channels are not reliable indicators of quality for *Andrographis* products. Significant variations in bioactive constituents arise from cultivation, harvest timing, and storage conditions, all of which directly influence the levels of AD and 14-DAD. Preparing herbal products from raw materials harvested at the optimal time should be utilized within one year to maintain the stability of active ingredients by minimizing degradation.

Therefore, quality assurance should standardize the raw materials and then determine the quality of product, both in-process control and finished product. All products should claim the stated amount of active content in the labeling. Specifically in *A. paniculata*, the quantitative analysis of a comprehensive profile of active marker compounds, rather than a single constituent, is essential to ensure both therapeutic effect and product safety.

CONCLUSION

This study developed and validated a cost-effective HPLC–PDA method, modified from the USP 43–NF 38, for the simultaneous quantification of four diterpene lactones in *Andrographis* products using AD as a single reference standard. The method complied with all validation criteria specified in ICH Q2(R2), demonstrating its reliability and suitability for routine quality evaluation. Analysis of fifteen registered products revealed significant variation in four diterpene lactones, with the quality of many products failing to comply with the USP43–NF 38 and THP 2021 standards. In addition, the potential degradation of AD into 14-DAD, a compound associated with vasorelaxant activity, under inappropriate storage conditions raises important safety concerns. These findings emphasize the need for a comprehensive quality control framework that should

monitor both AD and 14-DAD contents in all batches for the quality assurance of *Andrographis* products.

Appendix A. Supplementary data

Supplementary data associated with this article can be found at https://dx.doi.org/10.2306/scienceasia1513-1874.2026.056.

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Appendix A. Supplementary data

Table S1 The RRTs of four diterpene lactones, using AD as the reference peak, meeting the USP43–NF 38 criteria, and the percentage recovery for four diterpene lactones, using *A. paniculata* solution spiked with mixed standard.

Analyte	Lab-1		Lab-2		Relative	Peak area			%*R*
	Retention time	Relative Retention time	Retention time	Relative Retention time	retention time (USP43-NF38)	*A. paniculata* solution	Mixed standard	*A. paniculata* solution spiked with mixed standard	
AD	16.27	1.00	14.47	1.00	1.00	2,689,625	3,049,946	5,838,091	103.2
NAD	19.5	1.20	17.72	1.23	1.16	375,543	885,404	1,276,366	101.7
14-DAD	21.4	1.31	19.55	1.36	1.31	2,274,218	2,226,647	4,594,720	104.2
ADG	25.62	1.57	22.89	1.59	1.50	ND	1,225,131	1,199,296	97.9

ND = not detected.