

1-1-2021

Validated ultra-performance chromatographic method for simultaneous determination of andrographolide and its four related diterpenoids in *Andrographis paniculata* capsules

Jankana Burana-Osot

Panadda Phattanawasin

Uthai Sotanaphun

Chanokporn Sukonpan

Lawan Sriphong

See next page for additional authors

Follow this and additional works at: <https://digital.car.chula.ac.th/tjps>

 Part of the [Pharmacology Commons](#)

Recommended Citation

Burana-Osot, Jankana; Phattanawasin, Panadda; Sotanaphun, Uthai; Sukonpan, Chanokporn; Sriphong, Lawan; and Kumsum, Anchalee (2021) "Validated ultra-performance chromatographic method for simultaneous determination of andrographolide and its four related diterpenoids in *Andrographis paniculata* capsules," *The Thai Journal of Pharmaceutical Sciences*: Vol. 45: Iss. 1, Article 9. Available at: <https://digital.car.chula.ac.th/tjps/vol45/iss1/9>

This Article is brought to you for free and open access by the Chulalongkorn Journal Online (CUJO) at Chula Digital Collections. It has been accepted for inclusion in The Thai Journal of Pharmaceutical Sciences by an authorized editor of Chula Digital Collections. For more information, please contact ChulaDC@car.chula.ac.th.

Validated ultra-performance chromatographic method for simultaneous determination of andrographolide and its four related diterpenoids in *Andrographis paniculata* capsules

Authors

Jankana Burana-Osot, Panadda Phattanawasin, Uthai Sotanaphun, Chanokporn Sukonpan, Lawan SripHong, and Anchalee Kumsum



Validated ultra-performance chromatographic method for simultaneous determination of andrographolide and its four related diterpenoids in *Andrographis paniculata* capsules

Jankana Burana-Osot^{1*}, Panadda Phattanawasin¹,
Uthai Sotanaphun², Chanokporn Sukonpan¹,
Lawan Sriphong¹, Anchalee Kumsum¹

¹Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakhon-Pathom, Thailand, ²Department of Pharmacognosy, Faculty of Pharmacy, Silpakorn University, Nakhon-Pathom, Thailand

Corresponding Author:
Jankana Burana-Osot,
Department of Pharmaceutical
Chemistry, Faculty of
Pharmacy, Silpakorn
University, Nakhon-Pathom,
73000, Thailand.
Tel: +66 34255800,
Fax: +66 34255801.
E-mail: buranaosot_j@su.ac.th

Received: Jul 08, 2019
Accepted: Jan 13, 2020
Published: Jan 05, 2021

ABSTRACT

Introduction: *Andrographis paniculata* contained many pharmacologically active diterpenoids. **Objectives:** A simple and rapid isocratic reverse phase chromatographic method was developed using UPLC for simultaneous determination of 5 diterpenoids; andrographolide, deoxyandrographiside, neoandrographolide, 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide in *A. paniculata* capsules. **Materials and Methods:** The chromatographic separation was achieved on a Waters Acquity BEH C18, 50 × 2.1 mm, 1.7 μm column using mobile phase consisting of acetonitrile and water in a ratio of 25:75 (v/v). The flow rate was 0.4 ml/min with a runtime of 12 min. The method was validated according to the regulatory guidelines with respect to specificity, linearity, precision, accuracy, limit of detection, limit of quantitation and robustness. **Results:** The proposed method provided linear responses ($r^2 > 0.999$) with good precision (%RSD < 3%). The recovery values ranged from 98.00% to 101.36% for all compounds. **Conclusion:** A validated UPLC method for analysis of andrographolide and four bioactive analogs in *A. paniculata* was successfully established. The developed method was applied for analysis of 5 active compounds in capsule formulation.

Keywords: *Andrographis paniculata*, method validation, ultra-performance liquid chromatography

INTRODUCTION

Andrographis paniculata (Burm. f.) Wall. ex Nees (known as Fa-Tha-Lai in Thailand) is herbal medicine that widely planted in South and Southeast Asia. This plant has been used as a constituent in many formulas of *Ayurveda* traditional medicine since it has plentiful therapeutics array such as antipyretics, antidiarrheal, anti-inflammation in laryngitis, and treatment of mild upper respiratory tract infection.^[1-4] The Thai FDA also listed this medicinal plant and its formulation product such as capsule, pill, and tablet in the National List of Essential Medicines (NLEM) as herbal medicine for the treatment

of upper respiratory tract infection and diarrhea. The protocols for qualitative and quantitative analysis of *A. paniculata* crude drug and andrographis capsules (Fa-Tha-Lai capsules) are entitled in the Thai Herbal Pharmacopoeia (THP).^[3-4] According to the THP monograph, this medicinal plant contained many pharmacologically active diterpenoids including andrographolide, neoandrographolide, and 14-deoxy-11,12-didehydroandrographolide [Figure 1] and the major diterpenoid that used as a chemical marker for the assay of *A. paniculata* is andrographolide. In addition to the three compounds in THP, the other related diterpenoids, 14-deoxyandrographolide and deoxyandrographiside, with pharmacological activities were also found in *A. paniculata*.^[5-7]

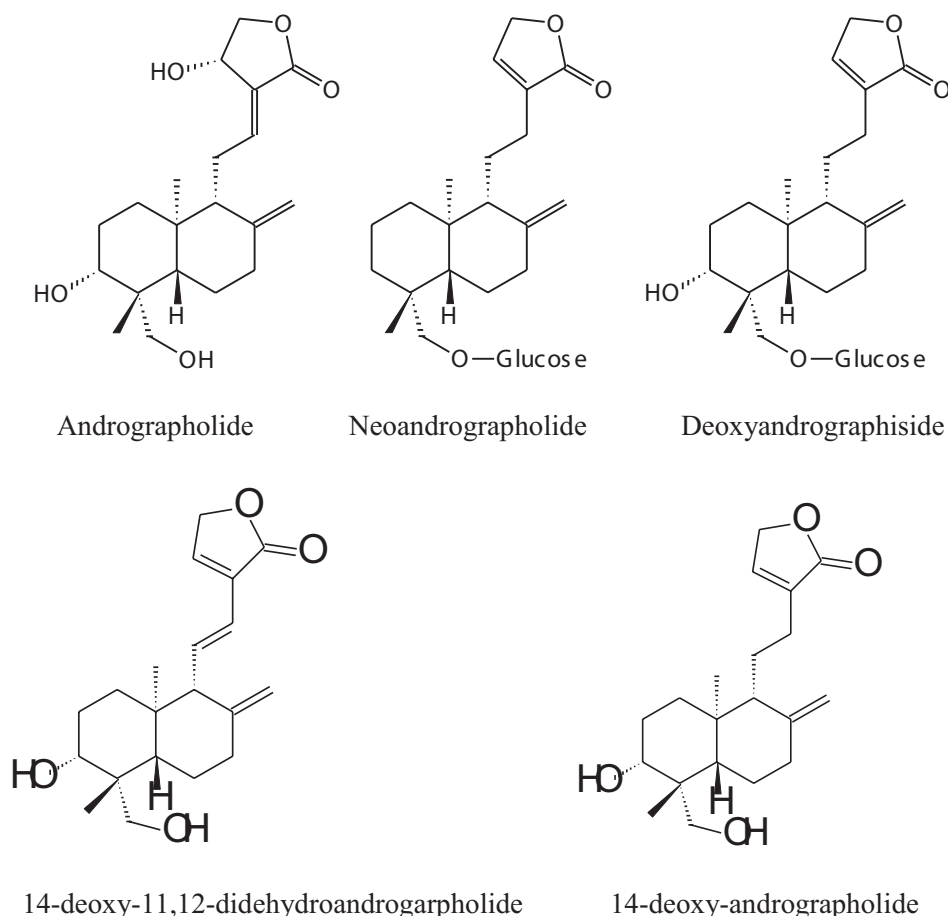


Figure 1: Chemical structures of andrographolide, neoandrographolide, deoxyandrographiside, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide

According to the THP assay monograph, the content of andrographolide in crude drug is determined by HPLC method.^[3-4] Several HPLC methods have been reported for the separation of andrographolide and its related diterpenoids.^[8-12] Since many andrographolide analogs and other natural diterpenoids exist in the plant, it is difficult to assay andrographolide and its related analogs separately within an optimal and short time. Moreover, in the USP42 assay monograph of crude drug and extracts, the amount of andrographolide in the existence of other three diterpene lactones is determined using a gradient HPLC method with 45 min run time.^[13] To speed up the assay, an ultra-performance liquid chromatography (UPLC) or rapid resolution liquid chromatography (RRLC) has been developed for analysis of these diterpenoids in *A. paniculata*.^[14] However, there is no report available for the validated HPLC or UPLC analytical method for *A. paniculata* in the present of deoxyandrographiside. Therefore, this study was aimed to develop an isocratic UPLC method for the determination of five active constituents including andrographolide, neoandrographolide, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, and deoxyandrographiside in *A. paniculata* capsule. The developed method was validated with respect to system suitability, specificity, linearity, precision, accuracy, limit of detection, limit of quantitation, and robustness.

MATERIALS AND METHODS

Chemical and Reagent

Five standard diterpenoids, andrographolide (95.4%), deoxyandrographiside (90.0%), neoandrographolide (90.7%), 14-deoxyandrographolide (91.4%), and 14-deoxy-11,12-didehydroandrographolide (93.1%), were prepared and purified from dry powder of the aerial part of *A. paniculata* following a procedure published previously.^[15] Five *A. paniculata* capsule formulations (brand 1–5) were purchased from local drugstore in Thailand.

Acetonitrile and methanol (HPLC grade) were purchased from Merck (Darmstadt, Germany). Other chemicals used were of analytical grade. High purity water was prepared using Milli-Q RO system (Millipore, Bedford, MA, USA).

Instrumentation and Chromatographic Conditions

The UPLC-PDA (photodiode array) system consisted of an Acquity UPLC H-Class separation module equipped with a binary solvent manager, autosampler system, and UV/visible detector (Waters Corporation, Milford, USA). The output signal was monitored and processed using Water Empower 3 software. A reversed-phase column, 100 × 2.1 mm i.d., packed

with 1.7 μm , Acquity UPLC BEH-C18 (Ethylene Bridged Hybrid) (Waters Corporation, Milford, USA) and an Acquity UPLC column in-line filter kit (0.2 μm stainless steel filter) were used. The chromatographic separation was carried out under isocratic elution with acetonitrile:water (25:75, v/v) at a flow rate of 0.4 ml/min. The mobile phase was filtered through a 0.2 μm polyvinylidene fluoride (PVDF) membrane filter and degassed by sonication before use. The wavelength was monitored at 223 nm, the column temperature was maintained at 40°C, and an injection volume of 1 μl was used. The PDA detector was operated in scan mode covering the range of 200–400 nm.

Preparation of Standard Solutions

Standard stock solutions of andrographolide, deoxyandrographiside, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide (1 mg/ml) were prepared individually in HPLC grade methanol. A combined standard solution containing andrographolide, deoxyandrographiside, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide in HPLC grade methanol was prepared (8 levels each) and filtered through a 0.2 μm PVDF membrane filter.

Preparation of Sample Solutions

The contents of five commercial capsules were weighed and mixed well. An aliquot of powder equivalent to the weight of 250 mg was accurately weighed into a 100 ml volumetric flask and added a part of methanol. After sonication for 30 min, the mixture was made up to volume with methanol and then filtered. Five milliliters of the filtrate were then pipetted into 10 ml volumetric flask and made up to volume with methanol and filtered through a 0.2 μm PVDF membrane filter.

RESULTS AND DISCUSSION

Development and Optimization of Method

Initially, C18 column for HPLC (75 \times 4.6 mm i.d., packed with 3.5 μm) was investigated. Two organic modifier, acetonitrile and methanol, were used for optimization. Acetonitrile gave better peak shape and higher resolution as compared to methanol. However, neither of two solvent could be well separated 14-deoxyandrographolide from 14-deoxy-11,12-didehydroandrographolide. Moreover, the run time was longer than 40 min.

Then, an UPLC C18 column (100 \times 2.1 mm i.d., packed with 1.7 μm) was studied subsequently. It was found that the selectivity was different when using acetonitrile instead of methanol. When using methanol, the order of elution was andrographolide, deoxyandrographiside, 14-deoxyandrographolide, 14-deoxy-11,12-didehydroandrographolide, and neoandrographolide, respectively, while neoandrographolide was eluted before 14-deoxyandrographolide and 14-deoxy-11,12-didehydroandrographolide when acetonitrile was employed in mobile phase. The mobile phase with methanol modifier could not completely separated 14-deoxyandrographolide

from 14-deoxy-11,12-didehydroandrographolide. Moreover, chromatogram of *A. paniculata* sample showed that deoxyandrographiside was not resolved from the other matrix in sample. Satisfactory separation was achieved when acetonitrile was used. The best separation was achieved at 40°C using mobile phase acetonitrile:water (25:75) in an isocratic mode. The run time was about 12 min. Using the UPLC system enabled to reduce the analysis time into one-third of the time spent in the conventional HPLC. The typical UPLC chromatogram of standard solution and *A. paniculata* sample is shown in Figure 2.

System Suitability

System suitability was achieved by injecting 10 replicates of five standard mixture solutions. Based on the chromatograms of standard preparations, the retention times of

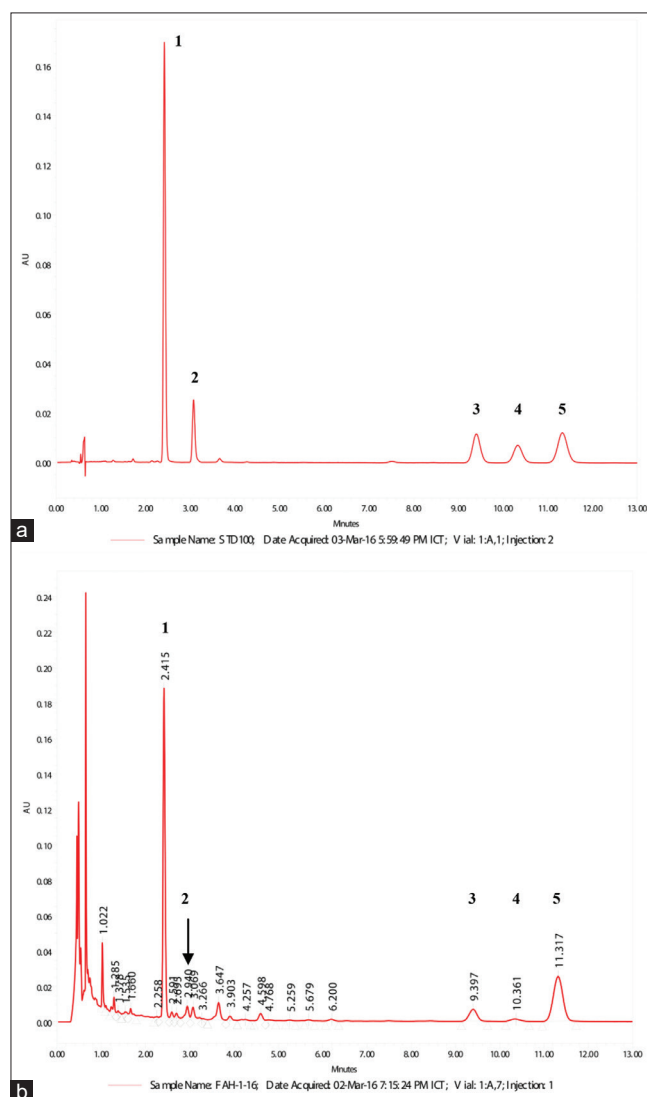


Figure 2: UPLC chromatograms of (a) standard mixing solution at concentration of 100 $\mu\text{g}/\text{ml}$ and (b) sample solution. Peaks; 1: Andrographolide, 2: Deoxyandrographiside, 3: Neoandrographolide, 4: 14-deoxyandrographolide and 5: 14-deoxy-11,12-didehydroandrographolide

Table 1: System suitability testing ($n = 10$) for the developed UPLC conditions

Compound	Retention time (Rt) (min)	%RSD of Rt	%RSD of peak area	Capacity factor (k')	USP tailing factor (T)	Number of theoretical plate (N)	Resolution (R)
Andrographolide	2.369	0.40	0.97	4.92	1.14	13753	6.52
Deoxyandrographiside	2.989	0.49	1.13	6.47	1.17	13783	6.68
Neoandrographolide	9.110	0.62	1.18	21.78	1.05	14199	2.74
14-Deoxyandrographolide	10.084	0.56	1.38	24.21	1.07	14281	2.98
14-Deoxy-11,12-didehydroandrographolide	11.054	0.59	1.28	26.63	1.07	14453	2.72
Suitability criteria		<1%	<2%	>2	<1.5		>2

Table 2: Linearity and sensitivity data

Compound	Range ($\mu\text{g/ml}$)	Linear regression equation	r^2	LOD ($n=10$)		LOQ ($n=10$)	
				S/N	$\mu\text{g/ml}$	$\mu\text{g/ml}$	%RSD
Andrographolide	50–1000	$y = 5547.1x - 889.68$	0.9998	3.3	0.1	0.25	3.56
Deoxyandrographiside	5–500	$y = 1091.5x - 703.43$	0.9997	3.9	0.5	1.0	3.82
Neoandrographolide	5–500	$y = 1396.1x + 3909.3$	0.9994	3.9	1.0	2.0	4.89
14-Deoxyandrographolide	5–500	$y = 2016.5x - 1849.2$	0.9999	3.9	1.0	2.0	4.94
14-Deoxy-11,12-didehydroandrographolide	5–500	$y = 4021.8x - 8004.2$	0.9994	3.9	1.0	2.0	4.41

andrographolide, deoxyandrographiside, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide were 2.37, 2.99, 9.11, 10.08, and 11.05 min, respectively. The system suitability parameters listed in Table 1 were satisfactory with good specificity.

Method Validation

Specificity

Specificity was demonstrated by separately injecting standard mixture solutions of the five diterpenoids and sample solution. Peak purity was verified using photodiode array detection and Empower 3 software.

Peak of minor impurities in standard solution and other matrix in sample was observed to separate from the main peaks. Resolution between main peaks and any other peak was greater than 2. The purity angle of five main peaks was less than purity thresholds, indicating that no coelution from any other matrix in sample was detected.

Linearity, LOD, and LOQ

All calibration curves were plotted on linear regression analysis of the integrated peak areas (y) versus concentration (x , $\mu\text{g/ml}$) of the diluted standard solution at eight different concentrations. These solutions were analyzed individually in six replicates for the establishment of the calibration curve. The correlation coefficient (r^2) was found to be greater than 0.999 for all standard components which indicates a high degree of correlation between selected concentrations and their respective peak areas [Table 2]. Therefore, linearity was verified.

The LOD and LOQ were assessed at signal-to-noise ratio of 3 and 10, respectively, by injecting a series of dilute standard solutions with known concentration. The precision ($n = 10$) was also estimated at LOQ level. The results are shown in Table 2.

Precision

Precision of the method was determined in repeatability and intermediate precision. Repeatability was assessed by analyzing the amount of each compound in test samples in six replicates. Intermediate precision was established on 3 consecutive days by two analysts ($n=18$). The %RSD was calculated and results are summarized in Table 3. These data revealed that the described method had an acceptable degree of precision.

Accuracy

The accuracy was assessed by recovery studies using standard addition method. The recovery was evaluated in five replicates ($n = 5$) at the three concentration and the mean percent recovery ($n = 15$) and %RSD were calculated for each added concentration.

The recovery for all compounds ranged from 98.00% to 101.36% and the %RSD were all <2%, as shown in Table 4. The results showed that the method enables high accurate simultaneous analysis of the five diterpenoid compounds.

Robustness

Robustness was performed by applying little deliberate changes of the following method conditions: (i) Mobile phase composition; organic composition: $\pm 2\%$, (ii) temperature: $\pm 2^\circ\text{C}$, (iii) flow

Table 3: Repeatability and intermediate precision data

Compound	Repeatability (n=6)						Intermediate precision (n=18)
	Analyst 1		Analyst 1		Analyst 2		
	Day 1 ^a	%RSD ^b	Day 2 ^a	%RSD ^b	Day 3 ^a	%RSD ^b	
Andrographolide	0.80±0.01	1.03	0.80±0.01	1.09	0.79±0.01	1.10	1.38
Deoxyandrographiside	0.25±0.00	1.71	0.25±0.00	1.21	0.25±0.01	2.59	1.91
Neoandrographolide	0.42±0.00	1.16	0.42±0.00	0.92	0.42±0.01	1.37	1.26
14-Deoxyandrographolide	0.08±0.00	2.68	0.07±0.00	1.60	0.07±0.00	3.32	2.95
14-Deoxy-11,12-didehydroandrographolide	0.73±0.01	0.98	0.74±0.01	1.14	0.73±0.01	1.25	1.13

^aAverage±standard deviation (n=6). Value expresses in %W/W of sample. ^b% Relative standard deviation

Table 4: Recovery data

Compound	Spiked standard (µg/ml)	Recovery ^a (%)	%RSD ^b
Andrographolide	100	98.77±0.71	0.72
	150	100.15±0.38	0.38
	200	99.36±0.51	0.51
	Mean recovery (n=15)	99.43±0.91	0.92
Deoxyandrographiside	25	98.42±0.75	0.76
	50	98.28±0.85	0.87
	75	98.60±0.73	0.74
	Mean recovery (n=15)	98.43±0.73	0.75
Neoandrographolide	25	99.01±0.98	0.99
	50	99.74±1.26	1.26
	75	100.52±1.97	1.96
	Mean recovery (n=15)	99.75±1.50	1.50
14-Deoxyandrographolide	25	98.50±1.58	1.61
	50	98.00±1.78	1.82
	75	100.47±1.04	1.04
	Mean recovery (n=15)	98.62±1.73	1.76
14-Deoxy-11,12-didehydroandrographolide	25	101.36±1.04	1.02
	50	100.13±0.96	0.96
	75	99.18±0.41	0.42
	Mean recovery (n=15)	100.23±1.21	1.21

^aAverage±standard deviation (n=5). ^b% Relative standard deviation

rate: ±0.1 ml/min, and (iv) detection wavelength: ±2–3 nm. Sample and standard solutions were analyzed for each change. Change was made to evaluate its effect on the method. Obtained data for each condition were evaluated by system suitability and calculating percent of content.

In all the deliberately varied chromatographic conditions, the chromatogram of standard solutions for system suitability test showed good separations. All parameter values were within suitability criteria.

The result of % content for the various method modifications is shown in Table 5. The change in the mobile phase composition and wavelength did not show any impact on the % content of each compound. However, the higher flow rate (0.5 ml/min) and temperature (42°C) effected significantly for the determination of andrographolide and

deoxyandrographiside. The results [Table 5] showed that the % content of these two compounds was more than 5% from those value obtained using specified conditions. These indicated that measurements were susceptible to variations in analytical conditions. Hence, the flow rate and temperature should be suitably controlled to ensure the reliability of the analysis.

Solution Stability

The short-term stability of the analytes was established for standard and sample solutions under conditions as prescribed in the method. The purpose of this procedure was to determine the time during which the standard and sample solutions remain stable. The solutions were tested at 0, 1, 3, 6, 12, 18, 24, 32, 40, and 48 h. All compounds were defined to be stable as showing peak area change <1% relative to the initial value.

Table 5: Robustness data

Method condition	% content (W/W) (n = 5)				
	Andrographolide	Deoxyandrographiside	Neoandrographolide	14-Deoxyandrographolide	14-Deoxy-11, 12-didehydroandrographolide
Mobile phase					
23% acetonitrile	0.80±0.01	0.25±0.01	0.41±0.01	0.08±0.01	0.73±0.01
25% acetonitrile*	0.80±0.01	0.25±0.00	0.42±0.00	0.08±0.00	0.73±0.01
27% acetonitrile	0.82±0.01	0.26±0.00	0.43±0.00	0.08±0.00	0.71±0.01
Flow rate					
0.3 ml/min	0.80±0.01	0.25±0.00	0.42±0.00	0.08±0.00	0.73±0.02
0.4 ml/min*	0.80±0.01	0.25±0.00	0.42±0.00	0.08±0.00	0.73±0.01
0.5 ml/min	0.91±0.00 ^a	0.27±0.01 ^a	0.43±0.00	0.08±0.01	0.74±0.01
Temperature					
38°C	0.78±0.01	0.25±0.00	0.41±0.01	0.08±0.01	0.73±0.00
40°C*	0.80±0.01	0.25±0.00	0.42±0.00	0.08±0.00	0.73±0.01
42°C	0.95±0.00 ^a	0.27±0.01 ^a	0.41±0.00	0.08±0.00	0.74±0.01
Wavelength					
220 nm	0.79±0.01	0.25±0.00	0.41±0.00	0.08±0.00	0.74±0.00
223 nm*	0.80±0.01	0.25±0.00	0.42±0.00	0.08±0.00	0.73±0.01
225 nm	0.80±0.00	0.24±0.00	0.42±0.01	0.08±0.01	0.72±0.01

*Conditions as specified in the method. ^aVariation of > 5% compared to the result obtained from the specified method conditions

Table 6: The content of five compounds in *A. paniculata* capsules samples

Sample	Content ^a (%W/W)				
	1	2	3	4	5
Compound					
Andrographolide	4.00±0.02	1.43±0.03	1.62±0.02	3.23±0.01	2.87±0.01
Deoxyandrographiside	0.41±0.01	0.33±0.03	0.12±0.01	0.37±0.01	0.37±0.01
Neoandrographolide	0.40±0.02	0.36±0.00	0.38±0.01	0.40±0.02	0.43±0.02
14-Deoxyandrographolide	0.66±0.01	0.55±0.01	0.71±0.01	0.42±0.01	0.38±0.01
14-Deoxy-11,12-didehydroandrographolide	0.58±0.03	0.47±0.02	1.01±0.00	0.56±0.01	0.48±0.01

^aAverage±standard deviation (n=3)

Analysis of Five Diterpenoids in Commercial Samples

The validated UPLC method was applied to analyze five active diterpenoid compounds in *A. paniculata* capsules samples. Three injections of each sample were performed to obtain the mean value and the SD value. Contents of the five diterpenoids in the samples are listed in Table 6. The results indicate that all the five compounds were detectable in each sample. The variation of the amounts among the five samples was found based on the quality of the herbal materials.

CONCLUSION

A simple and fast isocratic UPLC method was developed and validated for simultaneous analysis of andrographolide and other four bioactive analogs in *A. paniculata* capsules. The results from the validation parameters showed that this method met almost all validation criteria including specificity, linearity, accuracy, and precise. The robustness of the method would be ensured under the specified flow rate and column temperature.

Using of the developed assay, all of five bioactive diterpene lactones including andrographolide, deoxyandrographiside, neoandrographolide, 14-deoxyandrographolide, and 14-deoxy-11,12-didehydroandrographolide were completely separated and eluted out within 12 min. It was about ¼ of time that suggested by the USP assay method. The fast isocratic method leads to use and spoil less organic solvent.

The results demonstrated that the proposed method could be readily employed as a suitable qualitative and quantitative analysis for *A. paniculata* in capsule formulation.

ACKNOWLEDGMENTS

The authors would like to thank Faculty of Pharmacy, Silpakorn University, for provision of laboratory facilities.

REFERENCES

1. Poolsup N, Suthisisang C, Prathanturug S, Asawamekin A, Chanchareon U. *Andrographis paniculata* in the symptomatic treatment of uncomplicated upper respiratory tract infection: Systematic review of randomized controlled trials. *J Clin Pharm Ther* 2004;29:37-45.
2. State Pharmacopoeia Committee. Pharmacopoeia of People's

Republic of China (2010 Version). Beijing, China: People's Medical Publishing House; 2010.

3. Department of Medical Sciences, Ministry of Public Health. Fa-Tha-Li: In Thai Herbal Pharmacopoeia 2017. Bangkok, Thailand: The Agriculture Co-operative Federation of Thailand; 2017. p. 108-16.
4. Department of Medical Sciences, Ministry of Public Health. Fa-Tha-Li Capsules: In Thai Herbal Pharmacopoeia 2017. Bangkok, Thailand: The Agriculture Co-operative Federation of Thailand; 2017. p. 117-8.
5. Srivastava A, Himanshu M, Verma RK, Gupta MM. Chemical fingerprinting of *Andrographis paniculata* using HPLC, HPTLC and densitometry. *Phytochem Anal* 2004;15:280-5.
6. Chan WW, Lin BF. Isolation and identification of bioactive compounds in *Andrographis paniculata* (Chuanxinlian). *Chin Med* 2010;5:1-15.
7. Niranjana A, Tewari SK, Lehri A. Biological activities of Kalmegh (*Andrographis paniculata* Nees) and its active principles-a review. *Indian J Nat Prod Resour* 2010;1:125-35.
8. Xu T, Pan J, Zhao L. Simultaneous determination of four andrographolides in *Andrographis paniculata* Nees by silver ion reversed-phase high performance liquid chromatography. *J Chromatogr Sci* 2008;46:747-50.
9. Ding L, Luo XB, Tang F, Yuan BJ, Guo M, Yao SZ. Quality control of medicinal herbs fructus gardeniae, common andrographis herb and their preparations for their active constituents by high-performance liquid chromatography-photodiode array detection-electrospray mass spectrometry. *Talanta* 2008;74:1344-9.
10. Jain DC, Gupta MM, Saxena S, Kumar S. LC analysis of hepatoprotective diterpenoids from *Andrographis paniculata*. *J Pharm Biomed Anal* 2000;22:705-9.
11. Pholphana N, Rangkadilok N, Saehun J, Ritruethai S, Satayavivad J. Change in the contents of four active diterpenoids at different growth stages in *Andrographis paniculata* (Burm.f.) Nees (Chuanxinlian). *Chin Med* 2013;8:3-12.
12. Karioti A, Timoteo P, Bergonzi MC, Bilia AR. A validated method for the quality control of *Andrographis paniculata* preparations. *Planta Med* 2017;83:1207-13.
13. The United States Pharmacopoeial Convention. The United States Pharmacopoeia, The National Formulary 42nd Revision. Rockville, MD: The United States Pharmacopoeial Convention, Inc.; 2019. p. 4716-9.
14. Song YX, Lin SP, Jin Z, Qin JF, Jiang ZY. Qualitative and quantitative analysis of *Andrographis paniculata* by rapid resolution liquid chromatography/time-of-flight mass spectrometry. *Molecules* 2013;18:12192-207.
15. Phattanawasin P, Sotanaphun U, Burana-osot J. Thin-layer chromatography-image analysis method for the simultaneous quantification of andrographolide and related diterpenoids in *Andrographis paniculata*. *J Planar Chromatogr* 2014;27:140-4.