



Validated UV-Vis Spectrophotometric Determination of Andrographolide in Herbal Nano-Preparation

INDAH HAIRUNISA¹, MUHAMMAD DA'I², ERINDYAH RETNO WIKANTYASNING^{2,*},
ANDHIKA RIZKY GILANG MAHAPUTRA², NORMAIDAH NORMAIDAH³ and MOHD FADZELLY ABU BAKAR⁴

¹Faculty of Health and Pharmacy, Universitas Muhammadiyah Kalimantan Timur, Samarinda 75243, Indonesia

²Faculty of Pharmacy, Universitas Muhammadiyah Surakarta, Kartasura, Sukoharjo 57162, Indonesia

³Faculty of Mathematics and Natural Science, Universitas Lambung Mangkurat, Banjarbaru, 70714, Indonesia

⁴Faculty of Applied Sciences and Technology, Universiti Tun Hussein Onn Malaysia (UTHM), 84600 Muar, Johor, Malaysia

*Corresponding author: E-mail : erindyah.rw@ums.ac.id

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Determination of major bioactive compounds in polyphyto-formulation is important for production of standardized herbal products. A fast, simple and inexpensive method for detection and quantification of andrographolide concentration in nanoemulsion preparations containing a combination of *Andrographis paniculata* (Burm f.) Ness. and *Phyllanthus niruri* L. has been developed. Detection and quantification were carried out using UV-vis spectrophotometry analysis with picric acid reagent and NaOH (8:2) in methanol solvents, read at maximum wavelength 479 nm with 22 min of incubation time. Validation was done by determine the parameters such as linearity, intra and interday precision, accuracy, limit of detection (LOD) and limit of quantification (LOQ). The results obtained showed linearity with $r = 0.9945$ ($y = 0.0109x - 0.2066$) in the range of 30-80 $\mu\text{g/mL}$. The accuracy (recovery) varied in the range of 97.15 to 104.42 %. Percentage of relative standard deviation (% RSD) for precision and intermediate precision value were 3.23 and 3.02 % with LOD value 211 $\mu\text{g/mL}$ and the LOQ 705 $\mu\text{g/mL}$. As a conclusion, this method is suitable to detect andrographolide content in herbal nano-preparation.

Keywords: *Andrographis paniculata*, *Phyllanthus niruri*, Spectrophotometry, Andrographolide, Nanoemulsion.

INTRODUCTION

Validation is a concept to ensure that a method used to determine the quality of the medicinal ingredients and the right and available drug preparations proven effectiveness and reproducibility [1].

The large number of medicinal plants in Indonesia encourages the pharmaceutical industry to develop the new and advance formulations to be able to increase the bioavailability of medicinal plants based product. The most important thing in the formulation of a phytopharmaceutical product is that the product contain standardized phytochemical from medicinal plants. *Andrographis paniculata* (Burm f.) Ness. or locally known as Sambiloto in Indonesia has been traditionally used to treat various diseases. This plant has been shown to display anti-diabetic activity [2-5]. This plant also display potent antioxidants activity [6-8]. *Phyllanthus niruri* L. or locally known as Meniran

in Indonesia also has been used widely in India, China and Malaysia. *Phyllanthus niruri* is ethnobotanically used to treat hepatitis, dysentery and rheumatism. The activity of *Phyllanthus niruri* as a source of antioxidants has been scientifically shown [9], in addition to other activities such as antibacterial, anti-diabetic and anticancer [10-12].

Previous studies have been carried out to validate the content of andrographolide from *A. paniculata* extract. Da'i *et al.* [13] studied the validated method to determine *A. paniculata* poly-herbal mixture using RP-HPLC. Manasa and Suneetha [14] developed the method to determine the content of andrographolide levels using a UV-Vis spectrophotometer. The result of this study showed good validation results for andrographolide levels in polyherbal mixture.

The purpose of this study was to validate the method for determination of andrographolide levels in nanoemulsion preparations containing a combination of *Andrographis*

paniculata and *Phyllanthus niruri* extract using a UV-Vis spectrophotometer.

EXPERIMENTAL

Dried herb of *Andrographis paniculata* was bought from the local herbal shop, Solo, Indonesia, while *Phyllanthus niruri* was purchased from the wet market (Pasar Gede) Solo, Indonesia. *P. niruri* was then dried under indirect sunlight for 3 days.

Sample extraction: Dried *Andrographis paniculata* and *Phyllanthus niruri* plants were grounded into powder using dry blender to get a uniform size. To eliminate chlorophyll, the samples were then soaked with petroleum ether (Sigma-Aldrich) in the ratio of 1:5 for *A. paniculata* and 1:7 for *P. niruri*. Then the mixture was filtered and the residue was dried for overnight. Dried residue were macerated using 70 % ethanol (Merck) for 5 days, while stirring occasionally. Then the filtrate was evaporated with a vacuum rotary evaporator (Buchi) until thick extract was obtained.

Preparation of nanoemulsion: Nanoemulsion samples were prepared by mixing SNEDDS (self nanoemulsion drug delivery system) into water with a water and SNEDDS ratio (2:1). Then the solution was homogenized using a magnetic stirrer for 10 min at medium speed without heating.

Stock and working solutions: A solution of andrographolide stock was prepared at concentration of 500 $\mu\text{g/mL}$ (solution A). For accuracy study, a solution of andrographolide stock (in methanol) was prepared at concentration of 5 mg/mL (solution B). Solution C was prepared by mixing 1 % picric acid in water and 10 % NaOH in water. Ratio of picric acid and NaOH was 8:2. All the solutions were prepared fresh prior to experiments.

Optimization of experimental conditions

Determination of operating time: The operating time was determined by mixing 0.4 mL of solution A with 2.5 mL of solution C then made up the volume of 5 mL with methanol. The solution is read after every 2 min at 481 nm.

Determination of maximum wavelength: The maximum wavelength is determined by mixing 0.4 mL of solution A with 2.5 mL of solution C then made up the volume of 5 mL with methanol. The solution is read after every 2 min at 481 nm. The solution is read again after incubation for 22 min. From the results, maximum wavelength (λ_{max}) was found to be 479 nm.

Andrographolide standard curve: Andrographolide concentration was prepared at different concentration from 30 to 80 $\mu\text{g/mL}$. Then each concentration was analyzed using a UV-Vis spectrophotometer at operating time and maximum $\lambda_{\text{max}} = 479$ nm. The absorbance obtained is a linear regression equation with X axis as the concentration and Y axis as absorbance.

Preparation of sample of analysis: Nanoemulsion (20 μL) was mixed with 1980 μL methanol. The sample was left to incubate for 5 min. Then 0.5 μL of sample was mixed with 2.5 mL of solution C and made up the volume of 5 mL with methanol. After 22 min, the solution is read at $\lambda_{\text{max}} = 479$ nm using a UV-Vis spectrophotometer.

Preparation of *Andrographis paniculata* and *Phyllanthus niruri* extracts nanoemulsion: A 100 mg of *A. paniculata* and *P. niruri* extracts were mixed with 5 mL of methanol. The mixture was allowed to sonicated for 10 min. The sample was allowed to rest for 5 min, then supernatant is taken and filtered

using microphore. Then the sample (25 μL) was mixed with 2.5 mL of solution C and made up the volume of 5 mL with methanol. After 22 min, the solution is read at $\lambda_{\text{max}} = 479$ nm using a UV-Vis spectrophotometer.

Determination of validation parameters

Intra-day and inter-day precision: Intra-day precision was performed by reading six replicates at the same concentration in the same day. Inter-day precision was made by conducting experiment at different days. The acceptance criteria is the RSD value less than 5 %.

Linearity: Linearity was performed by reading from six different concentration in the range of 30-80 $\mu\text{g/mL}$. The acceptance criteria is the R value more than 0.98.

Accuracy: Accuracy is performed by standard addition method by adding 3 series of concentration levels, 80 % (596 $\mu\text{g/mL}$), 100 % (746 $\mu\text{g/mL}$), 120 % (895 $\mu\text{g/mL}$). Each series of levels were replicated thrice. The acceptance criteria is the recovery value in range 95-105 %.

Limit of detection (LOD) and Limit of quantification (LOQ): Andrographolide standard curve was prepared using different concentrations (30-80 $\mu\text{g/mL}$).

RESULTS AND DISCUSSION

Optimization of wavelength and operating time: Andrographolide in polyherbal formulation can be determined using spectrophotometry analysis based in the presence of chromophore and auxochrome groups in the compound. Therefore, it is necessary to add picric acid in alkaline condition to form andrographolide complex which is orange in colour (**Scheme-I**). From the optimization results a value of λ_{max} of 479 nm and operating time for 22 min (Fig. 1) were obtained.

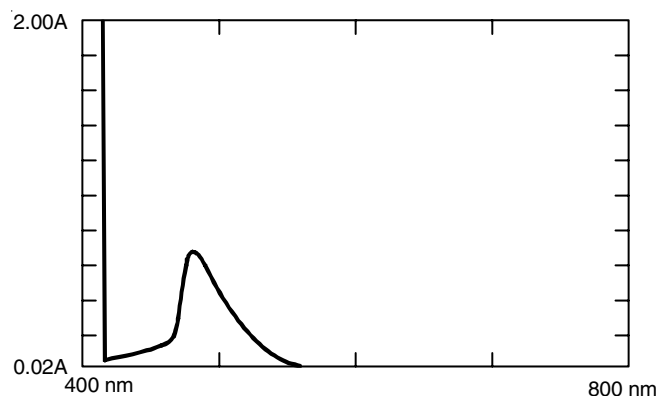
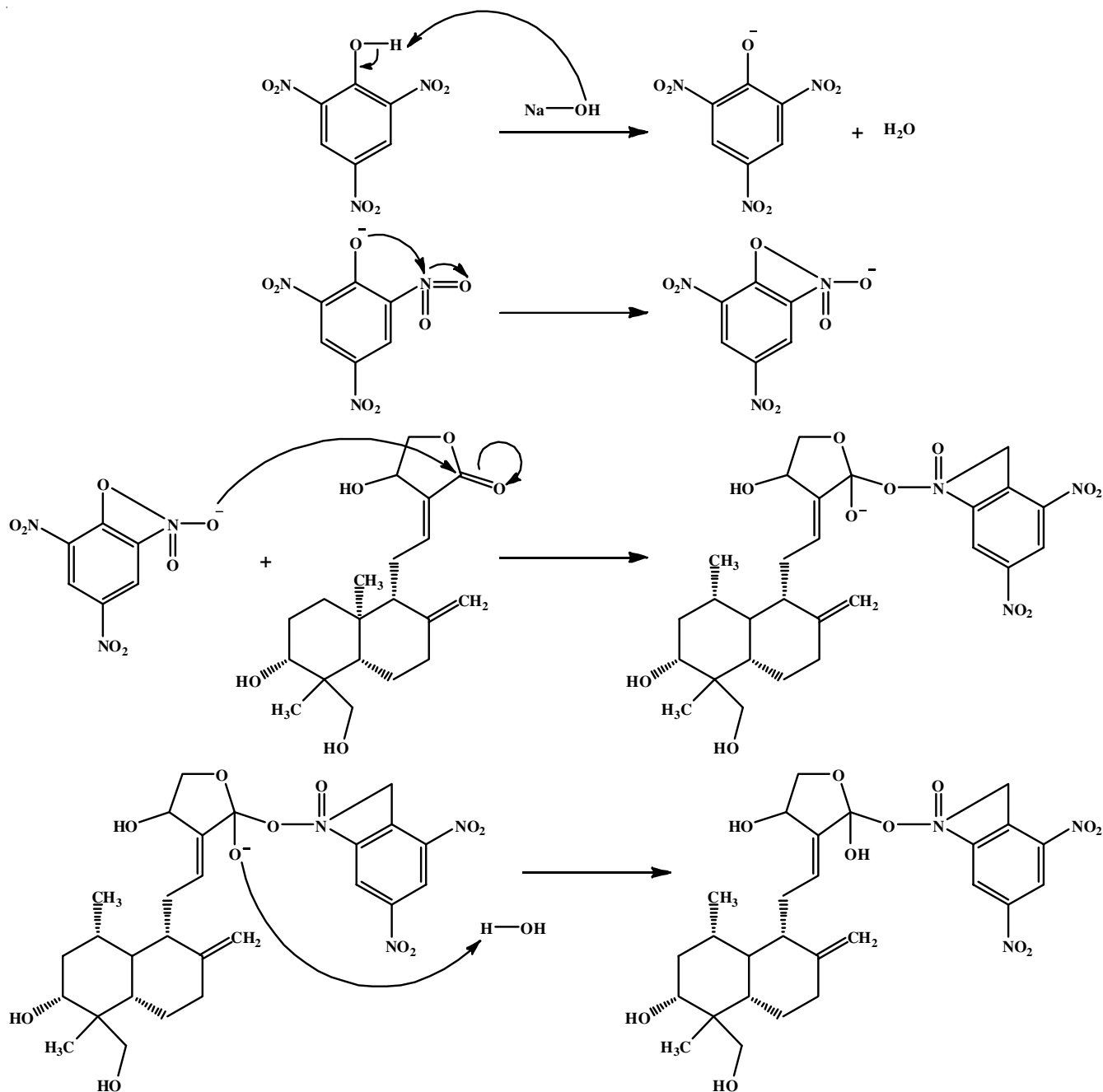


Fig. 1. Spectra of standard andrographolide absorption; Andrographolide level was analyzed using UV-vis spectrophotometer in a wavelength range of 400-800 nm. OT 22 min with λ_{max} 479 nm

Linearity: Linearity between the concentration of andrographolide and absorbance was calculated based on a series of concentrations of ranging from 30 to 80 $\mu\text{g/mL}$. The results are plotted between absorbance *versus* concentration and then a linear regression equation is made and the correlation coefficient value is obtained. From the experimental results, the linear regression equation is $y = 1.09 \times 10^2 - 2.07 \times 10^2$ with r value of 0.9945 (Fig. 2) [15].

Intra-day and Inter-day precision: Intra-day and inter-day precision of the experiment was conducted to validate the



Scheme-I: Mechanism of reaction for the formation of an andrographolide-picric complex

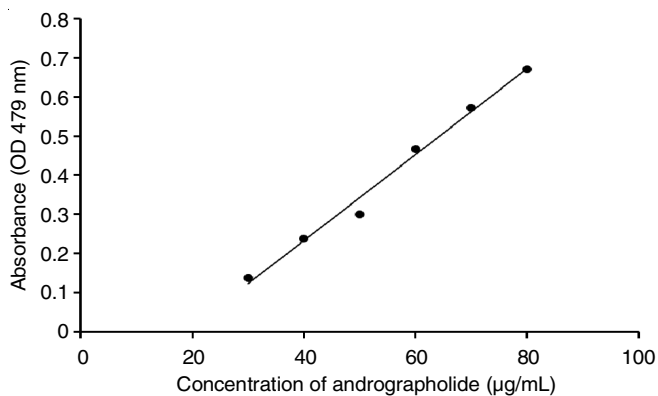


Fig. 2. Standard curve of andrographolide; The standard andrographolide curves were analyzed using a UV-vis spectrophotometer at a wavelength of 479 nm, 22 min OT, with a range of 30-80 µg/mL. Linear regression $y = 0.0109x - 0.2066$ with $r = 0.9945$

experiment. The results showed that the average level of andrographolide is almost similar between each experiment with the values of S.D. of 0.047 and 0.044, respectively (Table-1).

Accuracy: Evaluation of accuracy is based on percentage of recovery. The value of % recovery from three levels of concentration between is 97.15 to 104.42 % with % RSD value of 1.99-2.56 % (Table-2).

Limit of detection (LOD) and Limit of quantification (LOQ): The LOD and LOQ values are found to be 211.6 and 705.4 µg/mL. These results indicated that UV-Vis spectrophotometer method can detect andrographolide with the lowest concentration of 211.6 µg/mL, besides that it can quantify the andrographolide with the lowest limit of 705.4 µg/mL.

A study conducted by Manasa and Suneetha [14] with different reagents and conditions were almost similar with the

TABLE-1
INTRA- AND INTER-DAY PRECISION OF
ANDROGRAPHOLIDE

	Sample concentration (mg/mL)	Average \pm SD	RSD (%)
Intra-day precision	1.451	1.486 \pm 0.047	3.23
	1.439		
	1.549		
	1.441		
	1.508		
Inter-day precision	1.526	1.499 \pm 0.044	3.06
	1.489		
	1.512		
	1.453		
	1.526		
	1.563		
	1.448		

TABLE-2
ACCURACY RESULTS OF ANDROGRAPHOLIDE

Concentration level (%)	Analyte found (mg/mL)	Recovery (%)	Average \pm SD	RSD (%)
80	1.53	100.5	99.94 \pm 2.56	2.56
	1.51	97.15		
	1.54	102.08		
100	1.71	104.42	102.19 \pm 2.04	1.99
	1.69	101.74		
	1.68	100.4		
120	1.83	100.45	102.31 \pm 2.32	2.27
	1.84	101.56		
	1.87	104.91		

present experiments, which had the LOD and LOQ values of 1 and 3 $\mu\text{g/mL}$, respectively. This is due to the large standard concentration used therefore, the LOD and LOQ values are considered as not good. Based on the results, it can be said that the present method is valid for the determination of andrographolide levels, but it is less sensitive due to the limited LOD and LOQ values. The large LOD and LOQ values can be caused by several factors, besides the use of large standard concentrations, the sensitivity of tools used also affects the LOD and LOQ values which can influence the high LOD and LOQ values. Besides the intervention of other compounds can also interfere with the absorbance value obtained. In this method the compound which might interfere with the absorbance value is NaOH and picric acid.

Conclusion

In conclusion, a fast, simple and inexpensive method for detection and quantification of andrographolide concentration in nanoemulsion preparations containing a combination of *Andrographis paniculata* (Burm f.) Ness. and *Phyllanthus niruri* L. has been developed. The linear range of standard curve 30-80 $\mu\text{g/mL}$ with $r = 0.9945$ and the accuracy range at 97.15-104.42 % with RSD value is 2.27 % . The precision of intra-day and inter-day were 1.48 and 1.49 %, respectively. This validated method is expected to be a reference method for standardization of herbal nanopreparation.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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