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Research Article

Validation of A Simple HPLC-UV Method For the Quantification of Andrographolide in Self-Nano Emulsifying Drug Delivery System (Snedds) For Dissolution Study

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ABSTRACT

This research aim to validation of a simple, rapid and accurate HPLC-UV method for the quantification of andrographolide isolated from *Andrographis paniculata* Ness in Self Nano Emulsifying Drug Delivery System (SNEDDS) formulation during the dissolution test. The assay was performed using a XTerra® MS C18 column (150 mm X 4.6 mm, five µm) with a mobile phase of methanol and water (70: 30), at 0.8 mL/min flow rate and UV detection of 229 nm. Simulation gastric fluid (SGF) and intestinal fluid (SIF) were prepared as dissolution medium. The validation parameter was conducted including the test on linearity, precision, accuracy, LOD, and LOQ. The result showed an excellent linearity with r = 0.999 and good selectivity for both medium dissolution. The method showed sufficient precision, with a relative standard deviation (RSD) smaller than % Horwitz. The accuracy reported as % recovery was found to be 102.61 and 101.17 % in each SGF and SIF dissolution medium. LOD and LOQ were found 0.46 and 1.40 in SGF medium, 0.87 and 2.64 in SIF medium. In conclusion, the HPLC method developed showed specificity and selectivity with linearity in the working range, good precision and accuracy and suitable for quantification andrographolide in SNEDDS formulation.

Keywords: Validation, andrographolide, SNEDDS, HPLC.

INTRODUCTION

A bioactive substance named Andrographolide is found in *Andrographis paniculata* Nees (*A. paniculata*) that belongs to the family Acanthaceae massively growing in Asia's tropical areas[1]. This substance has various pharmacological activities, including anti-inflammatory, hepatoprotective, anti-viral, antithrombotic, antipyretic, hypoglycemic, anti-hyperlipidemia, and anti-cancer[2–4]. However, andrographolide has low oral bioavailability and poor water solubility. In addition, it has been a challenge for experts to develop a suitable *in vitro* dissolution test for poorly water-soluble drug products. The rate-limiting factor for oral absorption of substances with low solubility is the drug release[5,6].

Self Nano Emulsifying Drug Delivery System (SNEDDS) has been developed to improve the solubility, dissolution, and bioavailability of poorly soluble drugs in water such as lovastatin[7], nisoldipine[8], and glipizide[9]. Dissolution testing has been proven to be a valuable tool for evaluating the performance of Self-Nano Emulsifying Drug Delivery System (SNEDDS) in this formulation. Analytical method validation is aimed to verify the suitability of an analytical procedure of as a particular test for its proposed use. Regulations from Food and Drug Administration (FDA), including Good Manufacturing Practice (GMP) and Good Laboratories Practice (GLP), as well as such quality standard as International Organization for Standardization (ISO17025) recommend validation of analytical methods prior to and during a routine use[10]. Through a scientific study, method validation process proves whether an analytical method is appropriate for a certain purpose. The regulatory guidance for validation of method is provided by USP[11], and a draft document by the FDA contains latest guidelines for the development of methods and validation for methods of recent non-compendial testing[12].

Meanwhile, an analytical method must be validated to ensure the fulfillment of all the analytical application requirements and the result reliability. Consequently, the linearity, precision, specificity, accuracy, sensitivity, and quantification limit must be demonstrated in the test for analysis adequacy[13,14]. The objective of the present work is to develop a simple, rapid and accurate HPLC-UV for the quantification of andrographolide isolated from *Andrographis paniculata* Ness in Self Nano Emulsifying Drug Delivery System (SNEDDS) formulation for the dissolution test.

MATERIALS AND METHOD

Chemicals

Andrographolide was isolated from *Andrographis paniculata* base on our previous research[15], tween 20, polyethylene glycol 400 were purchase from Brataco Indonesia Ltd.; Capryol 90 was a gift from Gattefose, $Na_2HPO_2.2H_2O$ (Merck), KH_2PO_4 (Merck), HCl 37% pro analysis (Merck), methanol (pro-HPLC, J.T Baker).

Chromatographic conditions

The experiment used a high-pressure liquid chromatography (detector: Waters e2695 with UV-Vis

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Table 1: System suitability.

Parameters	Value	Acceptance criteria [12]
Capacity Factor (k')	2.37	> 2
Resolution (Rs)	3.65	> 2
Tailing Factor (T)	1.04	≤ 2
Theoretical plates (N)	2044	> 2000

2489). XTerra® MS C18 column (150 mm X 4.6 mm, 5 µm) was utilized to obtain separation with a mobile phase of methanol and water (60:40), 0.8 mL/min flow rate, and 229 nm UV detection. The Empower Program (Waters) was employed in the data analysis[16].

Preparation of standard solutions

To prepare 250 µg/mL concentration of the andrographolide stock standard solution, the shaking and sonication were performed using phosphate buffer pH 6.8 and HCl pH 3.4 as the media. Afterwards, the resulted stock solution was employed in the preparation of several mixed standard solution at certain concentrations.

System suitability

The system suitability test was performed to guarantee the appropriateness of chromatographic assay by evaluating the parameters that include resolution, tailing factor and theoretical plates. Then, a comparison was made between the evaluation results and the recommendation from the Center for Drug Evaluation and Research (CDER)[17].

Validation parameters

Method validation for dissolution test was performed using phosphate buffer pH 6.8 and HCl pH 3.4 as the media put in three different chambers. Following this procedure was a dissolution test of andrographolide SNEDDS preparation (30 mg/2 mL) using USP Apparatus 1 at a temperature of 37°±0.5°C and 100 rpm rotation speed in 900 ml medium solution. Sampling was done for each chamber by collecting 10 ml solution at minute 60 with 2-time replication. Samples were then filtered using a 0.45-µm and analyzed via HPLC.

Linearity

The stock solution was prepared with a series of a concentration of 10 ppm, 20 ppm, 30 ppm, 40 ppm, and 50 ppm using phosphate buffer pH 6.8 and HCl pH 3.4 followed by a calculation of the limit of regression coefficient (r), slope, and intercept.

Precision

Repeatability

Ten mL of solution was sampled and replicated six times from each dissolution medium at minute 15. They were filtered using a 0.45 µm membrane and analyzed using HPLC. The Standard Deviation, Coefficient of Variation, and % Horwitz were then calculated.

Intermediate

Intermediate precision was conducted on different days using the same equipment and laboratory for three replications. Then, sampling was done to obtain ten mL solution for six replications from both dissolution media at minute 15. The results were filtered using a 0.45 µm membrane, analyzed using HPLC, and calculated for their Standard Deviation, Coefficient of Variation, and % Horwitz.

Accuracy

Accuracy test was performed by adding a standard solution to the placebo to obtain 24, 30, and 36 ppm concentrations.

RESULTS AND DISCUSSION

System suitability

To identify the chromatography performance, a system suitability test was undertaken using three samples of the dissolution test, namely SNEDDS preparation, placebo sample, and andrographolide standard solution. A 0.45-µm filter was used to sieve the three samples followed by their injection. The system suitability was identified through several parameters, including capacity factor, resolution, tailing factor, and theoretical plates. The result of system suitability test is presented in Table 1.

Table 1 showed that the system suitability test had fulfilled the USP requirements. The capacity, resolution, tailing factor, as well as the number of theoretical plates, have met the provided criteria. Based on the result of placebo dissolution test, the inexistence of andrographolide area was marked by the absence of andrographolide retention time, which was at minute 2.7, compared to the standard andrographolide retention time and SNEDDS stock solution resulted from the dissolution test as illustrated in the following Figure 1.

Validation of Analysis Method

Andrographolide calibration curve in phosphate buffer pH 6.8 and HCl pH 3.5

The reading of several concentration intervals andrographolide concentration in phosphate buffer and HCl pH 3.5 were presented in Figure 2 and 3.

The regression test (Fig. 2 and 3) indicated that the concentration series andrographolide in phosphate buffer and HCl pH 3.5 showed 0.999 regression coefficient with the equation $y = 37746x + 46769$ and 0.999 with the equation $y = 49439x – 14408$. The ICH recommends that a good linearity value for analysis method validation should be more than 0.998. Therefore, the obtained linearity value has met the defined criteria. This regression result becomes the reference to determine the concentration level of accuracy, recovery, as well as precision testing.

Precision of phosphate buffer pH 6.8 and HCl 3.4

The result of precision is considered safe when the Coefficient of Variation (CV) value is smaller than the % Horwitz. The accuracy test for andrographolide quantification results in the values outlined in Table 2.

Precision test for the validation of andrographolide quantification has also been performed in both media with intermediate precision, which means that it is conducted on different days in three replications. From the test, the CV is compared to the % Horwitz, and it was found that the value is smaller than the % Horwitz. The acceptability criterion has therefore been fulfilled.

Accuracy Test in Phosphate Buffer pH 6.8 and HCl pH 3.4

The recovery test describes the response of the detector to the number of analytes added to the placebo. The value is stated in % recovery. The recovery value obtained from this study is presented in Table 3.

Table 2: The precision to quantification of andrographolide in buffer phosphate pH 6.8 and HCl pH 3.4 medium.

Day	Phosphate Buffer pH 6.8			HCl pH 3.4		
	Concentration (µg/mL)	CV (%)	% Horwitz	Concentration (µg/mL)	CV (%)	% Horwitz
1	28.91 ± 0.44	1.52	4.81	19.48 ± 0.35	1.78	5.10
2	26.15 ± 0.52	2.01	4.88	23.71 ± 0.62	2.60	4.95
3	30.55 ± 0.471	1.55	4.77	24.37 ± 0.33	1.37	4.93

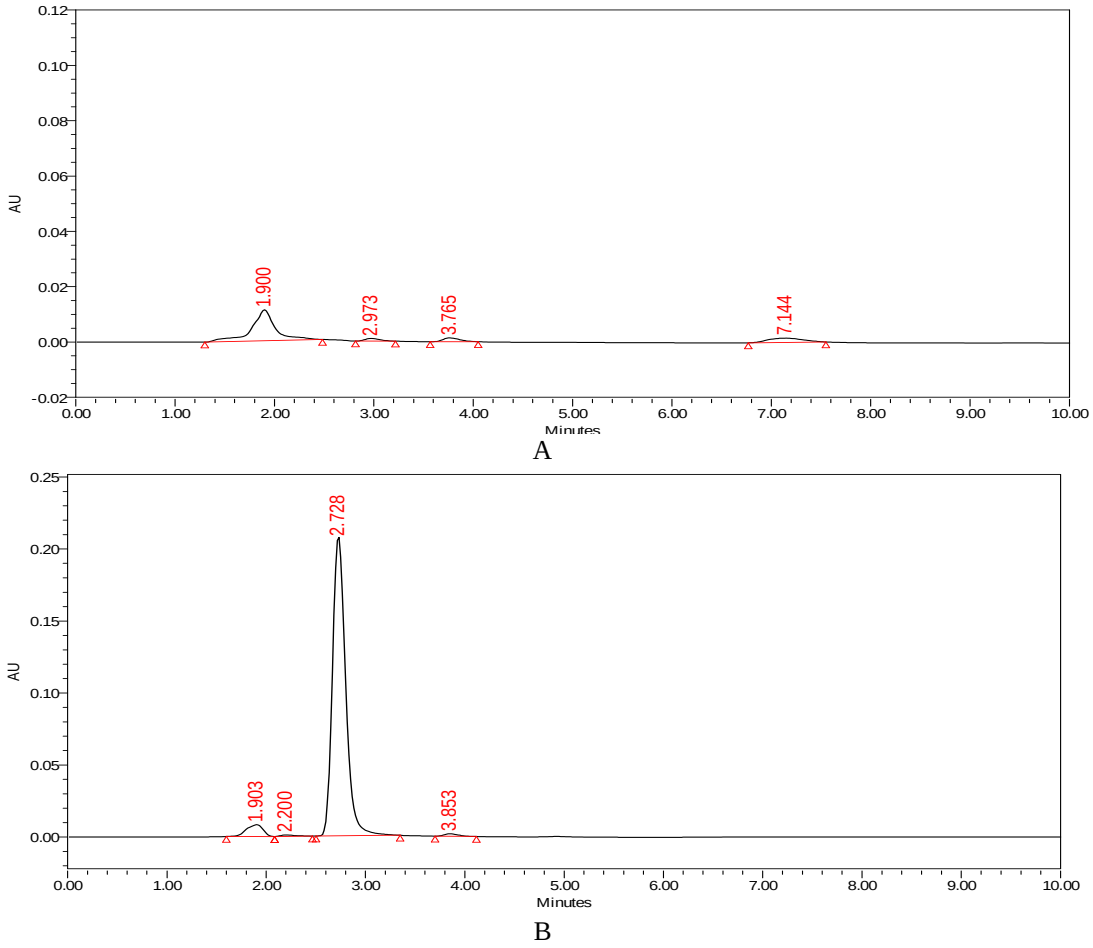

Figure 1: Placebo chromatogram (A) and andrographolide SNEDDS in HCl pH 3.4 (B).

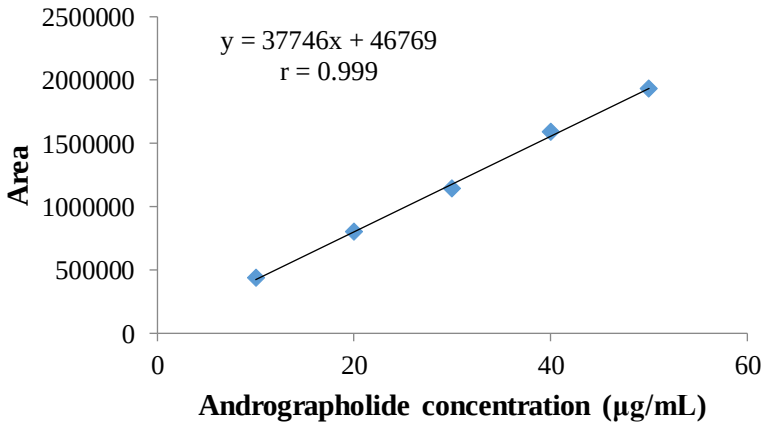

Figure 2: Andrographolide calibration curve in phosphate buffer pH 6.8.

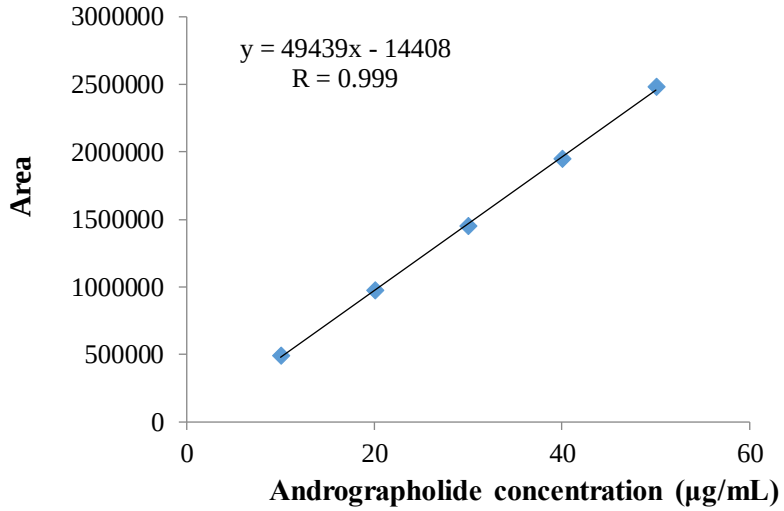

Figure 3: Andrographolide calibration curve in HCl pH 3.4.

Table 3: Accuracy Test in Phosphate Buffer pH 6.8 and HCl pH 3.4.

Concentration (%)	Recovery (%)	
	Phosphate buffer pH 6.8	HCl pH 3.4
80	102.28 ± 0.52	101.98 ± 0.19
100	103.02 ± 0.94	102.39 ± 0.12
120	102.52 ± 0.64	99.14 ± 0.13

Table 4: Determination of LOD and LOQ Values.

Medium	Parameter	Value obtained (µg/mL)
Phosphate buffer pH 6.8	LOD	1,95
	LOQ	5,92
HCl pH 3.4	LOD	1,95
	LOQ	3,13

In phosphate buffer pH 6.8, the mean recovery value for 24 µg/mL concentration is 102.28 ± 0.52 % and 103.02 ± 0.94 % for 30 µg/mL concentration, while the 36 µg/mL concentration has 102,52 ± 0.64 % recovery. Meanwhile, mean recovery value in HCl pH 3.4 for 24, 30 and 36 µg/mL concentration showed 101.97 ± 0.19; 102.39 ± 0.12 and 99.14 ± 0.13 % respectively.

According to the United States Pharmacopeia (USP), the recommended recovery value for dissolution test is 95-105%. It means the recovery values of the three concentrations obtained in this study have fulfilled the requirement from USP [18].

Determination of LOD and LOQ Values

The calculation of LOD (Limit of Detection) results in the lowest detectable concentration of a sample, while LOQ (Limit of Quantification) value states the smallest concentration of a sample to be accurately counted. The values of LOD and LOQ of each medium – phosphate buffer pH 6.8 and HCl pH 3.4 – are described in Table 4.

The theoretical values of LOD and LOQ from the linearity with HCl pH 3.34 are 1.95 ppm and 3.13 ppm. On the other hand, the theoretical values of LOD and LOQ from the linearity using phosphate buffer pH 6.8 are 1.95 ppm and 5.92 ppm. This parameter means that the samples have to reach a minimum concentration of 1.95 ppm in both HCl pH 3.4 and phosphate buffer pH 6.8 to be detectable, while 3.13 ppm in HCl pH 3.4 and 5.92 ppm in phosphate buffer pH 6.8 are the minimum concentration to be countable.

CONCLUSION

This study developed a simple, fast, and accurate HPLC method. The simplicity and high sensitivity of this technique have made it interesting especially for andrographolide quantification in SNEDDS for dissolution testing. In this research, the linearity has r = 0.9999 in both HCl pH 3.4 and phosphate buffer pH 6.8, while the accuracy in phosphate buffer pH 6.8 and HCl pH 3.4 is 102.28-103.02% and 99.14-102.39% respectively. Also, the precision is indicated by the % CV that is lower than % Horwitz.

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