

## EVALUATION OF PHYSICOCHEMICAL PROPERTIES OF A TRADITIONAL THAI ANTIHYPERTENSIVE HERBAL RECIPE IN VARIOUS PREPARATIONS

ARTHIMOND VUTTHIPONG<sup>1,\*</sup>, CHAOWALIT MONTON<sup>2</sup>, WORAWAN SAINGAM<sup>2</sup>, KINGKAN BUNLUEPUECH<sup>3</sup>, TOSSATON CHAROONRATANA<sup>1</sup>

<sup>1</sup>Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Lak-Hok, Muang, PathumThani, 12000, Thailand, <sup>2</sup>Sino-Thai Traditional Medicine Research Center, Faculty of Pharmacy, Rangsit University, Lak-Hok, Muang, PathumThani, 12000, Thailand, <sup>3</sup>Faculty of Traditional Thai Medicine, Prince of Songkla University, Hatyai, Songkhla, 90112, Thailand.  
Email: arthimond7@yahoo.com

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### ABSTRACT

**Objective:** The purpose of this article was to prepare a new suitable dosage form of a traditional Thai antihypertensive herbal recipe (TTAH). All details in a preformulation study and physicochemical properties of the preparations were reported.

**Methods:** Three different formulations were prepared and evaluated for their physicochemical properties. The preformulation studies were investigated including angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio before further formulation. The appropriate formulations were evaluated in their physicochemical properties such as weight variation, friability, thickness, hardness, disintegration time, and content uniformity.

**Results:** The C1 capsule contained 400 mg of herbal powder was the best dosage form because of its good results in physicochemical properties evaluation such as weight variation (489.2±10.84 mg), disintegration time (4.02±1.20 min), and content uniformity (piperine 98.38±1.78%, imperatorin 98.60±1.08%, and pinostrobin 102.44±1.29%).

**Conclusion:** Considering to formulation of the TTAH as a capsule, a tablet, or a pill, many parameters were evaluated. Pills and tablets failed on at least one criterion, with difficulties in manufacturing or disintegrating ability being recorded. Capsules passed all tested criteria and became the formulation of choice for TTAH.

**Keywords:** Traditional Thai antihypertensive herbal recipe, Physicochemical properties, Piperine, Imperatorin, Pinostrobin.

### INTRODUCTION

Altering paradigm in natural product development, many research interests had been shifted to the ancient knowledge of medication such as, traditional medicine (TM), and alternative medicine. Additionally, the World Health Organization (WHO) indicates an important of such the knowledge and has been promoted the use of TM [1]. Since then, TM becomes a hot issue; a lot of effort is put into its marketing, inversely to its safety and quality assessment. In 2005, TDR regulation about chemistry-manufacturing-control (CMC) of TM was launched in order to provide a brief guideline for TM evaluation [2]. In a quality control topic, it is indicated that a type of herbal product and its method of manufacture should be provided to support the clinical trial of TM. This report was associated with the data in the WHO guideline and ASEAN harmonization [3-4]. In Thailand, many traditional Thai medicine (TTM) has been widely used since now. In this article, a traditional Thai antihypertensive herbal recipe (TTAH), which was reported previously, was selected to be a model for formulation development. Recently, it was reported only a short discussion for TTAH preparation [5]. More details in the processes of formulation development and evaluation are needed [6]. Thus, an effort was made to prepare a suitable formulation of the antihypertensive herbal powder and to evaluate the physicochemical properties. All parameters were optimized in order to standardize the amount of active ingredients per dose, and to improve patient compliance. The information will be discussed in order to support the use of the TTAH and will be included in the chemistry-manufacturing-control (CMC) prior to clinical trials.

### MATERIALS AND METHODS

#### Materials

All herbal powders including *Acanthusebracteatus*, *Aeglemarmelos*, *Boesenbergiapandurata*, *Cyperusrotundus*, *Pipernigrum*, and *Tinosporacrispa* were purchased from CharoensukOsod, NakornPathom province, Thailand. Avicel PH 102, Aerosil®, and

magnesium stearate were purchased from ChagzhouKaide Import and Export Co., Ltd., China. Polyvinyl pyrrolidone K 30 was purchased from Sigma-Aldrich®(USA). HPLC grade acetonitrile, water, and formic acid were purchased from B&J (Korea). Piperine, imperatorin, and pinostrobin standards were purchased from Sigma-Aldrich®(USA). Individual stock solutions (1.0 mg/ml) of three standards were prepared in acetonitrile-water (80:20) and filtered through 0.45 µm membrane filter. Working standards, piperine (0.25, 0.5, 2.5, 5, and 10 µg/ml), imperatorin (0.01, 0.05, 0.1, 0.5, and 1 µg/ml), and pinostrobin (0.04, 0.2, 0.4, 2, and 4 µg/ml) were prepared by diluting the corresponding stock solution with acetonitrile-water (80:20) for LC-MS analysis.

#### Herbal pill preparation

The herbal powders were tested by preformulation studies; angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio. As presented in the traditional method, the herbal powder was mixed with honey, rolled into a spherical shape, and cut into small pieces. The formulation which prepared by this method is represented by P1.

#### Herbal tablet preparation

##### Direct compression method

Initially, herbal powder, avicel PH 102, and aerosil were mixed together by the geometric dilution method. Magnesium stearate was added and mixed together for 3 min. The ingredient ratios were shown in Table 1. The formulation which prepared by the direct compression method is represented by D1. The mixture powders were tested by preformulation studies; angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio before tablets compression.

##### Wet granulation method

Initially, herbal powder, avicel PH 102, povidone K30, and aerosil were mixed together by the geometric dilution method. Water was

then added into the mixture powders until a damp mass occurred, sieved through an 18-mesh sieve to produce granules. The granules were dried in hot air oven at 60 °C for 4 h. The dried granules were sieved again through a 20-mesh sieve and magnesium stearate was then added and mixed together for 3 min.

The granules were tested by preformulation studies; angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio before tablets compression. Then, the granules were compressed into tablets using a single punch tableting machine (Charatchai machinery Model: CMT 12, Thailand) with a die diameter of 10.3 mm. The ingredients ratio is shown in Table 1 which designed as W1. The tablets were tested for the physicochemical properties such as, weight variation, friability, tablets thickness, tablets hardness, disintegration time, and content uniformity.

#### Herbal capsule preparation

The herbal powders were tested by preformulation studies; angle of repose, bulk density, tapped density, Carr's index, and Hausner ratio before filled into capsules. 400 mg of herbal powders were filled into capsule no.0 using the manual capsule filling machine. This formulation was represented by C1. The capsules were tested for the physicochemical properties such as, weight variation, disintegration time, and content uniformity.

#### Preformulation studies

##### Angle of repose

The angle of repose was tested by the fixed funnel method. 5 g of powder mixture was poured into glass funnel. The lower tip of glass funnel was 5 cm height from the ground. The height (h) and radius (r) of pile were measured, and then calculated using the following equation (1).

$$\tan \theta = \frac{h}{r} \dots\dots\dots (1)$$

$\theta$  = angle of repose (°)

h = height (cm)

r = radius (cm)

##### Bulk density

7 g of powder mixture was accurately weighted and gently poured into 25 ml glass cylinder. The volume of powder mixture was recorded and calculated using the following equation (2).

$$\text{Bulk density} = \frac{m}{V_0} \dots\dots\dots (2)$$

m = mass (g)

$V_0$  = unsettled apparent volume (cm<sup>3</sup>)

##### Tapped density

The glass cylinder with powder mixture from bulk density testing was used to test tapped density. It was tapped using tapped density tester (Erweka D-63150, Germany) for 1,250 strokes. The volume of tapped powder mixture was recorded and calculated using the following equation (3).

$$\text{Tapped density} = \frac{m}{V_f} \dots\dots\dots (3)$$

m = mass (g)

$V_f$  = final tapped volume (cm<sup>3</sup>)

##### Carr's index

Data from bulk density and tapped density testing were used for calculate compressibility index using the equation (4).

$$\text{Compressibility index} = \frac{(V_0 - V_f)}{V_0} \times 100 \dots\dots\dots (4)$$

$V_0$  = unsettled apparent volume (cm<sup>3</sup>)

$V_f$  = final tapped volume (cm<sup>3</sup>)

##### Hausner ratio

Hausner ratio was calculated the equation (5).

$$\text{Hausner ratio} = \frac{V_0}{V_f} \dots\dots\dots (5)$$

$V_0$  = unsettled apparent volume (cm<sup>3</sup>)

$V_f$  = final tapped volume (cm<sup>3</sup>)

##### Physicochemical properties evaluation

All tablets, capsules, and pills which prepared by our colleagues were tested for their reasonable physicochemical properties.

##### Weight variation

Twenty tablets were individually accurately weighed. Each tablet weight was recorded. The weight variation was represented as mean ± SD in mg.

##### Friability

The tablets had any dust removed before testing. Ten tablets were accurately weighed together, and friability was tested using a friability tester (K.S.L. Engineering, Thailand). After 4 min of rotation at 25 rpm, any loose dust from the tablets was removed before accurately weighing again. If friability was not more than 1.0% it was considered acceptable. The friability was calculated using the following equation (6).

$$\text{Friability} = \frac{(W_{\text{before}} - W_{\text{after}})}{W_{\text{before}}} \times 100 \dots\dots\dots (6)$$

$W_{\text{before}}$  = weight of tablets before test (g)

$W_{\text{after}}$  = weight of tablets after test (g)

##### Thickness

Ten tablets were individually measured using the thickness tester (Mitutoyo Corp. Model: ID-C112TB Absolute, Japan). Results were reported as mean ± SD in mm.

##### Hardness

Ten tablets were measured using a hardness tester (Erweka D-63150 Model: TBH220TD, Germany). Results were reported mean ± SD in kilopond (kP).

##### Disintegration time (DT)

Six tablets were tested by a disintegration tester (K.S.L. Engineering, Thailand) following the United State Pharmacopeial method, and water was used as the disintegration medium at 37 °C. DT of each tablet was recorded in minutes. If DT was not more than 30 minutes it was considered acceptable.

##### Content Uniformity

To determine the amounts of three markers such as; piperine, imperatorin, and pinostrobin in the preparations, 10 samples of each TTAH dosage forms were crushed into small pieces (for any tablets or pills) and sonicated 5 times in 50 ml hexane for 20 min, pooled, and evaporated. The extracts were then analyzed using LC-MS method as described previously [7]. The uniformity of content was calculated separately to each analyzes and reported as mean ± SD in percentages compared to the established average amounts of each markers in TTAH [6]. The average values were 1.43 %w/w, 0.05

%w/w, and 0.30 %w/w for piperine, imperatorin, and pinostrobin, respectively.

**Table 1: Formulation of herbal tablets/capsule/pill**

Ingredients	D1	W1	C1	P1
Herbal powder	500	500	400	300
Avicel PH 102	143.5	130.5	-	-
Aerosil	-	6.5	-	-
Magnesium stearate	6.5	6.5	-	-
Povidone K30	-	6.5	-	-

## RESULTS AND DISCUSSION

TTAH is the herbal product. According to CMC evidence needed to support clinical trials, the form of product and its method of

manufacture are required. In this study, four preparations were prepared and compared in order to choose the suitable dosage forms for the recipe. The herbal powder and granule were evaluated in a pre-formulation study to optimize flow ability. The angle of repose results indicated flow ability of herbal powder was poor. This herbal powder was used directly in the preparation of P1 and C1. In D1, Avicel PH 102 and magnesium stearate were added into the formulation, the angle of repose of this formulation was not significantly different from P1 and C1.

The flow ability was improved in W1 in which Aerosil® was added into the formulation. Flow abilities of the materials for the preparations were in the following order: W1 > D1 > P1 = C1 (Table 2). The Carr's index and Hausner ratio which used to predict flow characteristics were optimized. The results were correlated to the angle of repose results in which the herbal powder showed poor flow characteristics while the herbal granule showed excellent flow ability (Table 2).

**Table 2: Preformulation study**

Parameters	D1	W1	C1	P1
Angle of repose	51	25	61	61
Bulk density	0.39	0.43	0.34	0.34
Tapped density	0.57	0.46	0.48	0.48
Carr's index	32	6	29	29
Hausner ratio	1.48	1.07	1.41	1.41

The physical appearances of tablet were brown, smooth and concave in shape, while pill was in a spherical shape. The physicochemical of all preparations was showed in Table 3 and Table 4. The weight variation of only D1, W1, and C1 was in acceptable range but W1 showed the best result which indicated good uniformity of the preparation. Friability and hardness was tested only for D1, W1, and P1. It was found that the friability of all preparations was less than 1%, revealing their stability against erosion during a process of drug shipping. The hardness results of these preparations were correlated to the friability results. In order to monitor the ability of dosage form to disintegrate before being dissolved and absorbed

into the blood circulation, disintegration study must be performed. The result showed that the disintegration times of all preparations except P1 passed the criteria. It was reasonable since the hardness of P1 is 19.50 mm (Table 3). The content uniformity is the experiment to quantify the amount of markers in each dosage form and to assess its consistency. The standard value amounts of markers (piperine, imperatorin, and pinostrobin) were reported previously and can be used to calculate the content uniformity of the preparations. The relative standard deviation was calculated and it showed that the amount of markers in D1, W1, and C1 were consistent (Table 4).

**Table 3: Physicochemical properties evaluation of TTAH dosage forms**

Parameters	D1	W1	C1	P1
Weight variation (mg)	660.50 ± 8.21	658.28 ± 2.83	489.20 ± 10.84	314.11 ± 40.80
Friability (%)	0.52	0.30	-	0.01
Thickness (mm)	6.44 ± 0.09	6.85 ± 0.06	-	7.19 ± 0.14
Hardness (mm)	6.01 ± 0.83	6.20 ± 0.70	-	19.50 ± 1.93
Disintegration time (min)	15.94 ± 5.33	10.80 ± 2.69	4.02 ± 1.20	> 30

**Table 3: Content uniformity of reference standards in TTAH dosage forms**

Content uniformity (%)	D1	W1	C1	P1
Piperine	98.05 ± 2.76	98.23 ± 1.10	98.38 ± 1.78	95.12 ± 5.26
Imperatorin	97.11 ± 2.14	98.51 ± 0.97	98.60 ± 1.08	96.11 ± 4.45
Pinostrobin	102.25 ± 1.83	102.05 ± 0.63	102.44 ± 1.29	100.04 ± 4.33

The C1 capsule contained 400 mg of herbal powder was the best dosage form because of its results in physicochemical properties evaluation such as weight variation (489.2±10.84 mg), disintegration time (4.02±1.20 min), and content uniformity (piperine 98.38±1.78%, imperatorin 98.60±1.08%, and pinostrobin 102.44±1.29%). This dosage form is easy to prepare and might be practical in manufacturing scale.

## CONCLUSIONS

When considering formulating the TTAH as a tablet, a capsule, or a pill, many parameters were evaluated. Pills failed on many criteria such as disintegration time and content uniformity.

Although tablets preparing by wet granulation method was a good choice of preparation determining based on all evaluated parameters, but difficulties in manufacturing process was placed. Capsules passed all tested criteria and became the formulation of

choice for TTAH. Finally, this data will be included as a part of the CMC evidence prior to clinical trials.

## CONFLICT OF INTERESTS

Declared None

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