The Thai Journal of Pharmaceutical Sciences

Volume 38
Issue 0 *supplement issue*

Article 37

1-1-2013

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KEYWORDS: Maha-solos, Tablet, Piperine, RP-HPLC

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INTRODUCTION

Anxiety disorders are prevalent and widely interspersed with sickness in the general public. There are several categories of anxiety disorders, including generalized anxiety disorder (GAD), social phobia, panic disorder, agoraphobia, phobias, post-traumatic stress disorder (PTSD), and obsessive compulsive disorder (OCD). The general symptoms of anxiety disorder include obsessive thoughts, nightmares, problems sleeping, cold or sweaty hands and/or feet, shortness of breath, dry mouth, nausea and dizziness that affects quality of life. These disorders are accepted as risk factors for many diseases, especially psychiatric diseases.

The treatments of anxiety disorders currently available include tricyclic antidepressants (TCAs), selective serotonin reuptake inhibitors (SSRIs), serotonin–noradrenergic reuptake inhibitors (SNRIs), and other atypical antidepressant drugs such as monoamine oxidase inhibitors (MAOIs) (Nemeroff, 2007). However, the efficacy of drugs is frequently unstable and many of them create side effects such as weight changes, drowsiness or sedation, insomnia, agitation, apathy, fatigue, dry mouth, gastrointestinal disturbances, headache, cognitive impairment, and sexual dysfunction (Kennedy, 2006). The alternative possible treatment is herbal medicines. Therefore, research and development of more effective drugs without or less side effects are required.

In Thailand herbal medicines to relieve disorders have been used for many years, and research for new pharmacotherapy from medicinal plants has progressed significantly in the last 10 years. Moreover, a growing number of herbal products have been introduced into treating psychiatric disorders. Clinical studies in humans have shown that treatment with variuos Thai traditional medicines (TTM), such as Cassod tree (or Thai copper pod), Foetid Cassia and Confederate Vine, have a therapeutic effect on insomnia and anxiety disorders.

Maha-solos, a TTM formula, has been used more than 100 years for anxiety. These formulas are composed of *Solanum seaforthianum* Andr., *Glycyrrhiza glabra* Linn., *Amomum krervanh* Pierre., *Mesua ferrea* Linn., *Piper nigrum* Linn., *Zingiber officinale* Roscoe., *Piper retrofractum* Vahl., *Cinnamomum verum* J. Presl., *Olax scandens* Roxb., *Nelumbo nucifera* Gaertn., *Dracaena lourieri* Gagnep., *Tarenna hoaensis* Pitard., *Ligusticum sinense* Oliv. cv.Chuanxiong Hort., *Saussurea lappa* Clarke., *Angelica sinensis* (Oliv.) Diels., *Dryobalanops aromatica* Gaertn and sugar in the same proportions. Among 16 medicinal plants, pepper (*Piper nigrum* Linn.) compose of piperine is the medicinally use for treat many diseases such as snake venom poisoning, anti-epileptic. Maha-solos is available in powder dosage form and is boiled in water before use. Since such side effects of this current therapy are harmful for patients, new Maha-solos formulations have been developed to reduce side effects. However, in case of Maha-solos is prepare by boiled, the original method of preparing herbal medicines in the practice of TTM. The composition of herbs within a boiled solution can be changed for the condition of the patient. However, these traditional preparations have some disadvantages, such as the time to prepare and the administration of a large volume of unpleasant tasting medicine that can be troublesome for patients. Therefore, the researcher is interested in developing and formulating Maha-solos into a modern formulation and validating RP-HPLC method for determination of piperine in formulation. This will provide a viable resolution for delivering active ingredients using a cost-effective technology.

MATERIALS AND METHODS

Materials Piperine was purchased from Sigma Chemical Co.(USA). 16 Medicinal plant: *Solanum seaforthianum* Andr., *Glycyrrhiza glabra* Linn., *Amomum krervanh* Pierre., *Mesua ferrea* Linn., *Piper nigrum* Linn., *Zingiber officinale* Roscoe., *Piper retrofractum* Vahl., *Cinnamomum verum* J. Presl., *Olax scandens* Roxb., *Nelumbo nucifera* Gaertn., *Dracaena lourieri* Gagnep., *Tarenna hoaensis* Pitard., *Ligusticum sinense* Oliv. cv.Chuanxiong Hort., *Saussurea lappa* Clarke., *Angelica sinensis* (Oliv.) Diels., *Dryobalanops aromatica* Gaertn and sugar were purchased from Charoensuk Osod, Nakorn pathom, Thailand. Pharmaceutical excipient: colloidal silicon dioxide, magnesium stearate, microcrystalline cellulose, sodium starch glycolate were purchased from TTK science, Bangkok, Thailand. The Solvent for HPLC: methanol and water (Labscan, Thailand) were also purchased from TTK science, Bangkok, Thailand.

Quantitative analysis for determination of piperine by high performance liquid chromatography (HPLC) Quantitative analysis of piperine was analysed using a high performance liquid chromatography (HPLC) method, coupled to a UV detector set to 343 nm. The HPLC system consisted of a quaternary pump system (Agilent, 1260 VL), autosampler (Agilent, 1260 TCC) and UV/VIS with diode array detector (Agilent, 1260 DAD VL). A reverse-phase Zorbax C-18 column 4.6 x 150 mm was eluted by using a mixture (30:70) of methanol and water as the mobile phase with a flow rate was set to 1.0 ml/min and the injection volume was 10 µl. Validation parameters of linearity, accuracy, and precision were confirmed for this method.

Application to Maha-solos tablet formulation Prepared Maha-solos tablet stock solution by weighed accurately 10 tablets and crushed with mortar and pestle to obtain fine powder. Dissolve 600 mg of powder in 25 ml methanol and sonicated for 15 min. The solution was filtered through Whatman filter paper no.1. Then, pipette out 2.3 ml solution from stock solution and diluted up to 10 ml with methanol. Finally, pipette out 1 ml solution from stock solution and diluted up to 10 ml with methanol (0.55 mg/ml). To determine the specificity of the analytical method, standard stock solution of piperine, placebo solution and Maha-solos solution dissolve in the same solution. Both solutions were filtered through 0.45 µm Nylon filter. Equal volumes (10 µl) of each sample were injected into the chromatograph by autosampler and peak areas were measured.

Preformulation study To estimate the suitability of mixture for formulation, a preformulation study that checked the physical properties of the medicinal plant and excipients was required. The results of the preformulation study provided data that necessary for formulation and manufacture of tablet. Preformulation study, angle of repose, bulk density, tapped density, compressibility index and Hausner ratio were determine using the appropriate techniques.

Formulation development of Maha-solos tablets

Table 1 Formula of tablet

Ingredients	Weight per tablet (mg)				Function
	F1	F2	F3	F4	
Medicinal plant	500	500	500	500	Active ingredient
Colloidal silicon dioxide	12	24	36	36	Adsorbent/Glidant
Magnesium stearate	1.5	1.5	1.5	1.5	Lubricant
Microcrystalline cellulose	86.5	74.5	62.5	38.5	Diluent
Sodium starch glycolate	-	-	-	24	Disintegrant
Total	600	600	600	600	-

All ingredients as shown in Table 1 will be mixed and compressed by direct compression using a single punch tablet machine to make a tablet formula. Humidity in the room will be controlled to be lower than 30%RH. Amount of piperine from tablets will be analyzed by validated RP-HPLC method.

Physical properties of Maha-solos tablets

The evaluation data composed of weight variation, thickness, hardness, friability, and disintegration.

RESULTS AND DISCUSSION

Quantitative analysis for determination of piperine by RP-HPLC

Validation method of RP-HPLC analysis Linearity was studied by preparing standard stock solution of piperine at different concentration levels. When the concentrations of piperine and its respective peak areas were subjected to regression analysis by least squares method, a good linear relationship (R^2 = 0.9997) was observed between the concentrations of piperine and the respective peak areas in the range 0.1-0.5 mg/ml. The regression equation was found to be y = *152988146x-293050.8667*, where y is the peak area and x is the concentration of piperine.

To ensure the accuracy and reliability of the method, recovery studies were carried out in triplicate at three concentration levels of test concentration. The recovery of piperine was found to be in the range of 99.19-100.89 %

The intra-day precision of the assay method was evaluated by carrying out six independent assays of piperine test samples against qualified reference standard on same day and these studies were also repeated on six consecutive days to determine inter-day precision. The percentage of RSD of six assay values was calculated. Intra-day and inter-day, % RSD of piperine was found 0.63 and 0.26, respectively.

Application to Maha-solos tablet formulation The chromatogram of Maha-solos solution was compared with chromatogram of standard and placebo. As a result, the chromatogram of placebo was not shown the peak area at 4.026 minutes as shown in Figure 1.

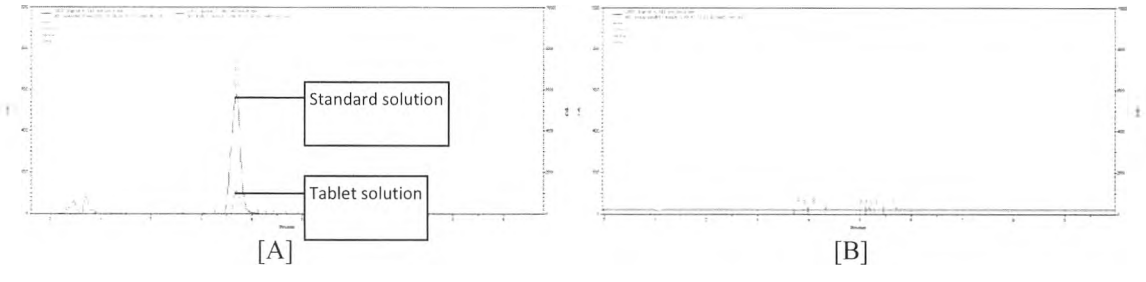

Figure 1 Chromatogram of piperine in [A] standard solution and tablet solution and [B] placebo solution

Preformulation study Preformulation study, angle of repose, bulk density, tapped density, compressibility index and Hausner ratio were showed in Table 2.

Table 2 Data of preformulaton study

Formulation	F1	F2	F3	F4
Angle of repose (°)	45.51	42.27	38.66	27.47
Bulk density (g/ml)	0.43	0.48	0.34	0.35
Tapped density (g/ml)	0.50	0.50	0.42	0.53
Carr's index (%)	14.00	4.00	19.05	33.96
Hausner ratio	1.16	1.04	1.24	1.51

Formulation development of Maha-solos tablets The formula of F1, F2, F3 and F4 contained medicinal plant at the amount 500 mg or 83.33% by weight. Colloidal silicon dioxide was individually tested for Maha-solos powder adsorption by mixing with excipients at the concentration of 2.0%, 4.0% and 6.0% by weight (F1-F3). The effect of adsorbent on physical properties of Maha-solos tablets was visually observed. It was found that colloidal silicon dioxide at the concentration of 6.0% by weight (F3) was the most suitable adsorbent for Maha-solos powder. F1 and F2 showed poor flow and exhibited sticking with

punch and die set. F3 formulation which contained 6.0% of colloidal silicon dioxide exhibited good flow, good compressibility and did not stick to punch and die set. Disintegretion time of F3 is 10.10±1.76 min. Then, we added sodium starch glycolate 4.0% by weight (F4) to decrease disintegration time to 5.88±0.89 min. The physical appearance of tablet of F3 and F4 were brown, round shape and smooth surface. The evaluation data of weight variation, thickness, hardness, friability, and disintegretion were showed in Table 3.

Table 3 Data of physical properties

Physical properties	F3	F4
Weight variation (mg)	585.99±11.48	589.40±7.35
Thickness (mm)	6.02±0.07	5.97±0.06
Hardness (kP)	7.00±1.26	7.58±1.02
Friability (%)	0.20	0.21
Disintegration (mins)	10.10±1.76	5.88±0.89

CONCLUSIONS

The analytical RP- HPLC method was developed and validated carefully for determination an amount of piperine. The developed method was simple and responsive which could be employed for quantity analysis of formulation. Maha-solos tablets are prepared successfully by direct compression method. Colloidal silicon dioxide can effectively adsorb Maha-solos powder and other excipients. The formulation overcomes the problems associated with hygroscopic excipients like medicinal plant.

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