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DISSOLUTION PROFILE OF A TRADITIONAL THAI ANTIHYPERTENSIVE HERBAL RECIPE IN VARIOUS PREPARATIONS

Arthimond Vutthipong[1,*], Worawan Saingam[2], Tanate Panrat [3], and Tossaton Charoonratana[1]

[1]Department of Pharmacognosy, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand, 12000.
[2]Sino-Thai Traditional Medicine Research Center, Faculty of Pharmacy, Rangsit University, Pathum Thani, Thailand, 12000.
[3]Prince of Songkla University International College, Prince of Songkla University, Hatyai, Songkhla, Thailand, 90110.

[*]Corresponding author : E-mail : arthimond7@yahoo.com

Abstract: A dissolution test was developed for a traditional Thai antihypertensive herbal recipe (TTAH) in various preparations. Three different formulations, such as pill, tablet, and capsule, were prepared and evaluated for their release profile in which piperine was chosen as a marker in dissolution study. The test was performed using paddle method with 50 rpm and dissolution medium composed of 0.2 M phosphate buffer pH 7.4. The sampling times were 5, 10, 15, 30, and 60 min. Liquid chromatography mass spectrometry (LC-MS) was used as a tool to determine amount of piperine. Different piperine release profiles were achieved and it was found that the reported method in this article was suitable for dissolution study only in capsule and tablet preparations.

Keywords: Dissolution, Paddle method, LC-MS, TTAH, Piperine

บทคัดย่อ : งานวิจัยนี้เป็นการศึกษาการปลดปล่อยของตัวยาในตำรับยาสมุนไพรไทยสูตรยาลดความดันที่เตรียมในรูปแบบต่างๆ 3 รูปแบบ คือ ยาลูกกลอน ยาเม็ด และยาแคปซูล โดยตัวยาที่ใช้ในการติดตามการปลดปล่อย คือ piperine การทดลองนี้ใช้วิธีการทดลองแบบใบพาย หรือที่เรียกว่า paddle method ที่ความเร็วรอบ 50 rpm และใช้ตัวกลางในการละลายเป็น 0.2 M phosphate buffer pH 7.4 จากนั้นทำการสุ่มเก็บตัวอย่างสารละลายที่เวลา 5, 10, 15, 30 และ 60 นาทีตามลำดับ ตัวอย่างทั้งหมดจะถูกวิเคราะห์หาปริมาณ piperine ด้วยเทคนิคโครมาโทกราฟีของเหลวสมรรถนะสูงและแมสสเปกโทรมิเตอร์ ซึ่งได้ข้อสรุปว่าวิธีการที่ใช้ในรายงานฉบับนี้มีความเหมาะสมที่จะใช้ศึกษาการปลดปล่อยของตัวยาในตำรับยาสมุนไพรไทยสูตรยาลดความดันในรูปแบบที่เป็นยาแคปซูล และยาเม็ด

คำสำคัญ: Dissolution, Paddle method, LC-MS, TTAH, Piperine

INTRODUCTION

Since a traditional medicine (TM) is in extensive use all through the world, many organizations have published announcements for supporting clinical trials of herbal products (WHO, 2013). Applying chemistry-manufacturing-control (CMC) to the production is one of the important issues which guarantee a good quality and the level of trust in the herbal products (TDR, 2005). There was a publication about a traditional Thai antihypertensive herbal recipe (TTAH) describing about the possibility to develop standardized TM (Charoonratana, 2014a). Previous report was also concerned about the physicochemical properties of TTAH which attempted to evaluate a suitable dosage form of TTAH (Vutthipong, 2014). In addition, from TDR guidance in year 2005, it was suggested to include dissolution data of the herbal products into the information needed to support a clinical trial. Dissolution evaluation is a standardized method for measuring the rate of drug release from the dosage form. In vitro data generated from dissolution experiment can be

related to in vivo pharmacokinetics data, thus providing the information of therapeutic effectiveness during product development (Kortejarvi, 2006). Since TTAH is herbal medicine which is a complex mixture and the dissolution study was designed for studying on the release of pure drug compounds, the problem was addressed. This issue was later discussed and it was accepted to use representative compounds determination in herbal product for the dissolution study, for an example, determination of curcuminoids in Khamin Chan capsule (THP, 2011). To support prior physicochemical data, in this study, the dissolution profile of all dosage forms such as pill, tablet, and capsule of TTAH was evaluated. The dissolution data between formulations had been compared and the correlation to physicochemical data had been made in order to support the CMC prior to phase 1 clinical trials.

MATERIALS AND METHODS

1) *Materials*:

All herbal powders including *Acanthus ebracteatus*, *Aegle marmelos*, *Boesenbergia pandurata*, *Cyperus rotundus*, *Piper nigrum*, and *Tinospora crispa* were purchased from Charoensuk Osod, Nakorn Pathom province, Thailand. HPLC grade acetonitrile, water, and formic acid were purchased from B&J (Korea). Piperine, KH_2PO_4, and NaOH were purchased from Sigma-Aldrich® (USA). A dissolution apparatus model SR_2 64-700-046 was from Hanson Research, USA. LC-MS system was from HPLC Dionex Ultimate TM^{3000} coupling with a MS Bruker Amazon SL.

2) *Methods*:

Drug preparation

Only 3 dosage forms, including pill, capsule, and wet granulated tablet, were used in drug release evaluation. First, as presented in the traditional method, the herbal pill was prepared by mixing the powder with honey, rolled into a spherical shape, and cut into small pieces. Second, for wet granulation, 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. 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. Third, for capsule, 400 mg of herbal powders were filled into capsule no.0 using the manual capsule filling machine.

Dissolution test

The test was conducted according to USP 24 Apparatus 2 guideline or paddle method with modification (USP, 1999). Since this experiment was the first observation of TTAH dissolution profile, the dissolution medium used in this study was 0.2 M potassium phosphate buffer pH 7.4. The buffer preparation was described; first, 27.22 g of monobasic potassium phosphate (KH_2PO_4) was dissolved in water, and diluting with water to 1000 mL, second, aliquot 250 mL of the KH_2PO_4 solution was transferred into a 1,000 mL volumetric flask, 195.5 mL of NaOH was added, and diluting with water to 1000 mL. The dissolution medium was degassed for 10 min. A 900 mL portion was transferred to a 1,000 mL dissolution vessel. The medium was maintained at 37 ± 0.5 °C for 30 min prior the test. The paddle speed was set to 50 rpm. Ten mL of samples were removed from the dissolution medium at 5, 10, 15, 30, and 60 min time intervals to analyse for piperine content.

Piperine quantification

Stock solution (1.0 mg/ml) of piperine standard was 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) was prepared by diluting the corresponding stock solution with acetonitrile-water (80:20) for LC-MS analysis. The LC-MS method was the same as in previous report (Charoonratana, 2014b). Briefly, the analysis was performed in gradient mode using aqueous 0.2% formic acid-acetonitrile from 60:40 to 20:80 (0-15 min), an isocratic at a ratio of 20:80 (15-20 min), and re-equilibrate at 60:40 (20-25 min). The column was maintained at 25^{o}C with a flow rate of 0.15 ml/min. ESI-MS evaluation was performed using a full scan in positive mode recorded on a mass range of *m/z* 100-1,000. The piperine content in 100 mg TTAH powder was analysed by LC-MS after TTAH extraction process which using 5 times sonication in hexane as described in previous paper (Charoonratana, 2014b).

RESULTS AND DISCUSSION

The purpose of this study was to determine the dissolution profile of TTAH in various preparations and figured out an appropriate marker for dissolution study. From the chromatograms, it was suggested to use piperine as a marker (Fig.1). There were four reasons that piperine was chosen as a marker for dissolution study. First, piperine is soluble in water. Second, only one marker quantification was sufficient in a quality control process of herbal medicine according to WHO guideline (TDR, 2005). Third, piperine itself contained chromophore and it was found to present in very high amount in TTAH, in which, can be easily applied to detect by HPLC or TLC in near future. Last, using piperine as the standard is profitable since the price is cheap. The standard curve of piperine was created and showed high degree of correlation with coefficient of determination (r^2 = 0.9997).

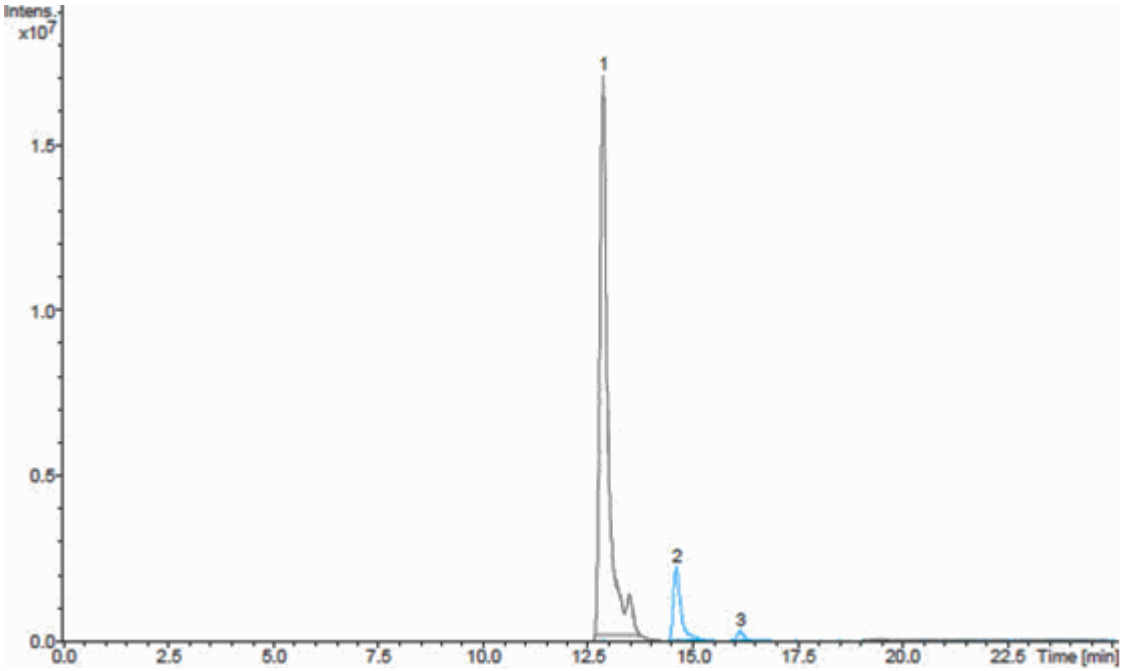

Figure 1. The chromatogram showed high amount of piperine (1)

In this experiment, the piperine content in TTAH powder was set as 1.5% w/w. The release of piperine from TTAH was observed using a modified USP paddle method, where the paddle speed was set at 50 rpm. The ranking order of release rate was from fastest to

slowest as capsule>tablet>pill (Fig.2). Comparison between dosage forms, an increase degree of dissolution was correlated to a decrease degree of disintegration time and hardness. The dissolution condition was proved to provide appropriate medium, agitation, time and temperature, since 85-95% release of piperine was achieved within 60 min for capsule and tablet (Fig.1). In contrast, the appearance of pill in the dissolution medium after 60 min is almost the same as before testing. In addition, it was previously reported that pill showed the greatest integration time and hardness. This information may be used to explain the event of the lowest piperine release from pill in which was detectable only at 60 min time point of testing.

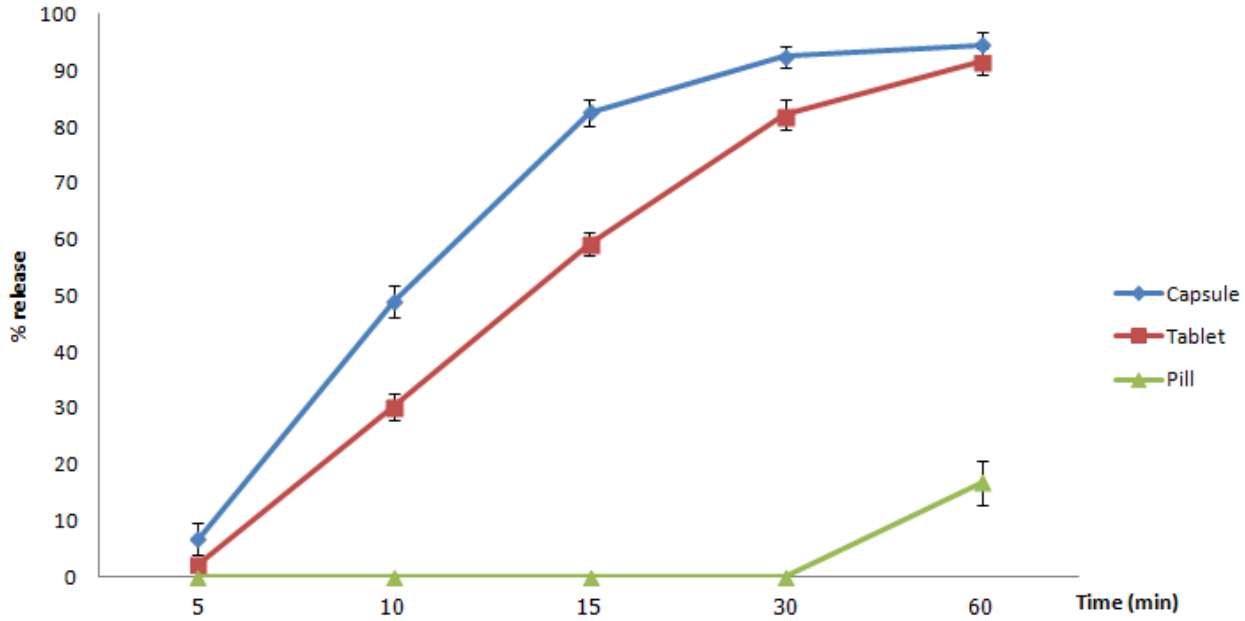

Figure 2. Dissolution profile of TTAH in various preparations using the paddle method at 50 rpm and 900 mL of phosphate buffer pH 7.4 at 37 ± 0.5 ^{o}C

CONCLUSION

Considering using capsule or tablet as dosage form of TTAH, piperine can be used as marker in dissolution test. For pill, a new developed dissolution procedure should be reconsidered. This article provided different dissolution profiles in various dosage forms which may be related to different bioavailability of TTAH. The dissolution study is simply and helpful method in which the information can be included in CMC prior to in vivo study in human.

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