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## Original Article

# Quantitation of curcuminoid contents, dissolution profile, and volatile oil content of turmeric capsules produced at some secondary government hospitals



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## ARTICLE INFO

## Article history:

Received 16 July 2015

Received in revised form

10 December 2015

Accepted 21 January 2016

Available online 23 February 2016

## Keywords:

curcuminoids

dissolution profile

turmeric capsules

volatile oil content

## ABSTRACT

The aim of this work was to validate the simple and rapid isocratic reversed phase-high performance liquid chromatography using a C-18 column for the determination of curcuminoid contents, dissolution profile, and volatile oil content of turmeric capsules produced at three secondary government hospitals. The validated reversed phase-high performance liquid chromatography method for three curcuminoids (bisdemethoxycurcumin, demethoxycurcumin, and curcumin) had a good linearity ( $R^2 > 0.9990$ ), accuracy (% recovery was 99.96–101.14%, 97.42–102.23%, and 98.01–99.12% for bisdemethoxycurcumin, demethoxycurcumin, and curcumin, respectively), precision (% relative standard deviation  $< 2\%$  and  $< 5\%$  for intraday and interday precision, respectively), including limit of detection, limit of quantitation, and system suitability. We found that turmeric capsules had a higher content of curcumin than bisdemethoxycurcumin and demethoxycurcumin. The total curcuminoid contents of all lots ranged from 12.02%w/w to 14.36%w/w. Dissolution profiles of curcuminoids were fitted with Higuchi model. Moreover, volatile oil content, determined using the hydrodistillation method, ranged from 7.00%v/w to 8.00%v/w. In conclusion, all nine lots of turmeric capsules from three secondary government hospitals met the standard criteria of the Thai Herbal Pharmacopoeia in the topic of curcuminoid contents, dissolution, and volatile oil content.

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<http://dx.doi.org/10.1016/j.jfda.2016.01.007>

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## 1. Introduction

Turmeric or *Curcuma longa* L. (Zingiberaceae) is an edible and herbaceous plant and has been used in Thai traditional medicines for a long time due to its many pharmacological activities. Turmeric rhizome contains curcuminoid compounds (2–6%), volatile oil (3–7%), fiber (2–7%), mineral matter (3–7%), protein (6–8%), fat (5–10%), moisture (6–13%), and carbohydrate (60–70%) [1]. The common volatile oils found in turmeric rhizome are  $\gamma$ -turmerone (38%),  $\alpha$ -turmerone (19%), and  $\beta$ -turmerone (15%) [2]. The major active compounds of turmeric rhizome are curcuminoids including bisdemethoxycurcumin, demethoxycurcumin, and curcumin. The chemical structures of these three curcuminoids are shown in Figure 1.

Turmeric rhizome powder had many pharmacological effects such as the treatment of irritable bowel syndrome [3], peptic ulcer [4], and inflammatory bowel disease [5]. There was a clinical trial in 2001 that studied 45 patients with peptic ulcers [4]. The patients received 600 mg of turmeric rhizome powder-filled capsules five times per day. The results showed that the peptic ulcers in 19 patients were absent within 12 weeks, peptic ulcers in 18 patients were absent within 8 weeks, and peptic ulcers in 12 patients were absent within 4 weeks. Results from blood chemistry and hematology of all patients showed no significant difference both before and after treatments [4]. Furthermore, in 2004, turmeric rhizome extract was studied in 207 patients with irritable bowel syndrome. They were administered one to two tablets (72–144 mg) of turmeric rhizome extract for 8 weeks. Data showed that the prevalence of irritable bowel syndrome decreased as well as abdominal pain and discomfort score [3]. In addition, curcumin has anti-*Helicobacter pylori* activity. However, this effect has not yet been clarified [6].

In Thai traditional medicines, turmeric is used as an anti-flatulent and for the treatment of peptic ulcers due to its richness of volatile oil and curcuminoid content. A turmeric rhizome powder-filled capsule is one of many official herbal products in the 2013 National List of Essential Medicines (Herbal Medicines) of Thailand [7]. Nowadays, turmeric capsules are the most popular herbal products compared with other herbal products. Moreover, some secondary government hospitals produced turmeric capsules and dispensed them to their patients. However, the quality control of this herbal product is rarely evaluated. The determination of curcuminoid contents, dissolution profiles, and volatile oil content of turmeric capsules is necessary to evaluate the quality of turmeric capsules. The purpose of this study was to validate a simple and rapid reversed phase-high performance liquid chromatography using a C-18 column in order to determine the individual and total curcuminoid contents, as well as dissolution profile of curcuminoids in turmeric capsules produced at some secondary government hospitals. In addition, volatile oil content was investigated.

## 2. Methods

### 2.1. Materials

Standard curcuminoids ( $\geq 98\%$ , high performance liquid chromatography (HPLC)) were purchased from Fluka Chemika, Buch, Switzerland as a mixture of bisdemethoxycurcumin, demethoxycurcumin, and curcumin. Samples of turmeric capsules (500 mg/capsule) were obtained from three secondary government hospitals with three lots each. Hospitals in the northern, the southern, and central Thailand were represented as Hospital A, B, and C, respectively. Nine lots of turmeric capsule samples that were produced within the previous 3 months were included in this work. Sodium lauryl

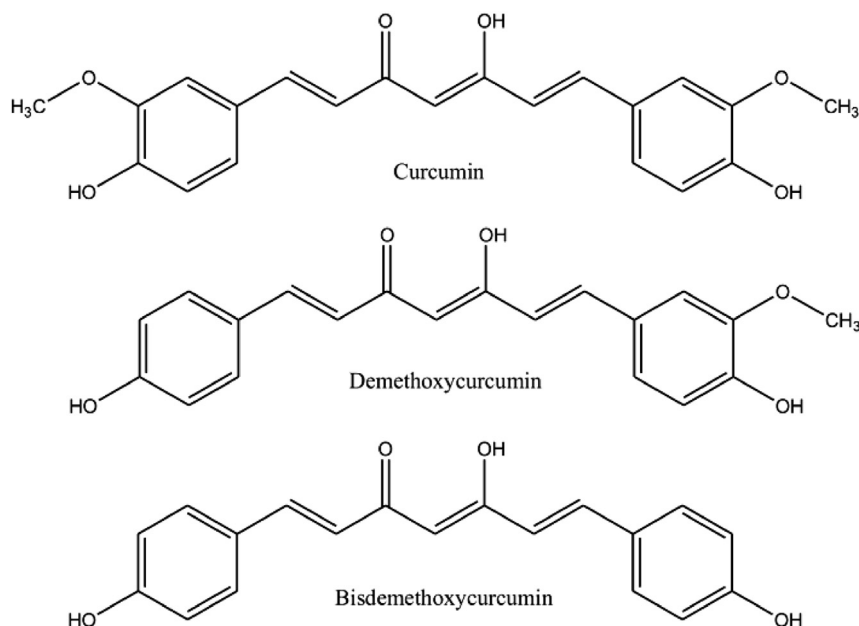


Figure 1 – Chemical structure of curcumin, demethoxycurcumin, and bisdemethoxycurcumin.

sulfate was purchased from Changzhou Kaide Import and Export Co. Ltd., Jiangsu, China. Tetrahydrofuran, glacial acetic acid, and hydrochloric acid were purchased from Carlo Erba, Val de Reuil, France. Acetonitrile (HPLC grade) and methanol (AR grade) were purchased from Burdick and Jackson, Seoul, Korea. Ultrapure water was produced by Puris-Expe UP water system (Model: Expe-UP Ele-M, Mirae ST Co., Ltd., Gyeonggi-do, Korea).

## 2.2. Standard curcuminoid solution preparation

Stock solution of standard curcuminoids was prepared in methanol at a concentration of 1 mg/mL. Stock solution was diluted to six concentrations and analyzed using the HPLC instrument (Agilent Technologies, California, USA). The calibration curves of three curcuminoids were constructed.

## 2.3. HPLC conditions

Analysis was performed on a HPLC instrument, an Agilent 1260 series that is equipped with a photodiode array detector and autosampler. Data analysis was performed using OpenLab CDS EZChrom software (Agilent Technologies, California, USA). The isocratic separation was done on an ACE C18-PFP column (250 mm × 4.60 mm internal diameter, 5 µm) (Advanced Chromatography Technologies Ltd., Aberdeen, Scotland). The column temperature was controlled at 33°C. The mobile phase consisted of 2%v/v acetic acid aqueous solution and acetonitrile in the ratio of 40:60 v/v with flow rate of 1 mL/min. The injection volume was 10 µL. The quantitation wavelength was detected at 425 nm.

## 2.4. Method validation

### 2.4.1. Linearity

Stock curcuminoid solution was diluted into six concentrations in methanol to obtain 0.16 µg/mL, 0.3 µg/mL, 0.6 µg/mL, 1.25 µg/mL, 2.5 µg/mL, 5.0 µg/mL, 10.0 µg/mL, and 20.0 µg/mL calculated as 0.019–0.600 µg/mL of bisdemethoxycurcumin, 0.053–3.400 µg/mL of demethoxycurcumin, and 0.250–8.000 µg/mL of curcumin. Each concentration was performed in triplicate. The standard solution was filtered through 0.22-µm membrane filter, and then injected into the HPLC instrument. The linear equation and  $R^2$  were reported for each curcuminoid compound.

### 2.4.2. Specificity

The specificity was observed by scanned UV spectrum of standard curcuminoids (wavelength range, 200–800 nm) in the beginning, middle, and end of each curcuminoid peak. Specificity was reported when the same spectrum of an individual peak in the three regions was found. In addition, the blank sample was injected into the HPLC instrument to confirm the specificity.

### 2.4.3. Precision

The three concentration levels of three curcuminoids were selected to study precision, both intraday and interday. Different concentrations of curcumin were analyzed in triplicate: (1) 0.075 µg/mL, 0.150 µg/mL, and 0.300 µg/mL of

bisdemethoxycurcumin; (2) 0.106 µg/mL, 0.213 µg/mL, and 0.425 µg/mL of demethoxycurcumin; and (3) 0.500 µg/mL, 1.000 µg/mL, and 2.000 µg/mL. The tests, performed on the same day and on three different days, were reported as intraday and interday precision, respectively. The relative standard deviation (%RSD) of each curcuminoid was reported.

### 2.4.4. Accuracy

The accuracy was tested using a spike method. Three curcuminoid concentration levels were added to known amounts of sample solutions. Each concentration was performed in triplicate. The percentage recovery of each curcuminoid was reported.

### 2.4.5. Limit of detection and limit of quantitation

The limit of detection (LOD) and limit of quantitation (LOQ) were evaluated based on signal-to-noise ratio. The standard curcuminoid solutions were diluted in methanol and injected into the HPLC instrument. The LOQ and LOD were reported when the signal-to-noise ratio was 10 and 3, respectively.

### 2.4.6. System suitability

The bisdemethoxycurcumin (0.038 µg/mL), demethoxycurcumin (0.213 µg/mL), and curcumin (1.000 µg/mL) solutions were injected into the HPLC instrument in six replicates. The retention time, peak area, theoretical plates, and asymmetry were reported as mean, standard deviation, and %RSD.

## 2.5. Determination of individual and total curcuminoid contents

Determination of curcuminoid contents was modified from the Thai Herbal Pharmacopoeia [8]. Briefly, turmeric powder was removed from 10 capsules and mixed together. Turmeric powder was weighed accurately at 300 mg, adjusted to 10 mL by tetrahydrofuran, and then shaken at ambient temperature for 24 hours. The supernatant was diluted 1250 times with methanol, filtered through a 0.22-µm membrane filter, and then injected into the HPLC instrument. Both individual and total curcuminoid contents were calculated. All samples were performed in triplicate.

## 2.6. Determination of the dissolution profile of curcuminoids from turmeric capsules

Determination of the dissolution profile of curcuminoids was modified from the Thai Herbal Pharmacopoeia [9]. Six turmeric capsules were sampled for the dissolution study. The study was carried out using Apparatus 1 (basket apparatus; Hanson Research Corp., California, USA.) at 100 rpm. The dissolution medium was 0.8%w/v sodium lauryl sulfate in 0.05M hydrochloric acid (900 mL) and the temperature was set at 37°C. The dissolution medium should be freshly prepared and used within 12 hours. The dissolution medium was sampled at 5 mL for 15 minutes, 30 minutes, 45 minutes, 60 minutes, and 90 minutes and the same volume of fresh medium was replenished. The sampling medium was diluted 50 times with methanol, filtered through a 0.22-µm membrane filter, and then injected into the HPLC instrument to

determine the dissolved curcuminoids. The dissolution profile of total curcuminoids was constructed.

### 2.7. Determination of volatile oil content

Turmeric capsules were sampled for at least 20 capsules. Turmeric powder was removed from the capsules and mixed together. Volatile content determination was performed using hydrodistillation following the Thai Herbal Pharmacopoeia. Ten grams of turmeric powder was added to a 500-mL round bottom flask and 100-mL water was added as a distillation liquid. Distillation apparatus was connected to the round bottom flask, then, a reflux condenser was inserted into the distillation apparatus. Water was filled to the graduated tube of the distillation apparatus to the standard line. The liquid was then heated and distillation was performed for 5 hours. At a predetermined time, the volume of volatile oil in the graduated tube of distillation apparatus was read and recorded [10].

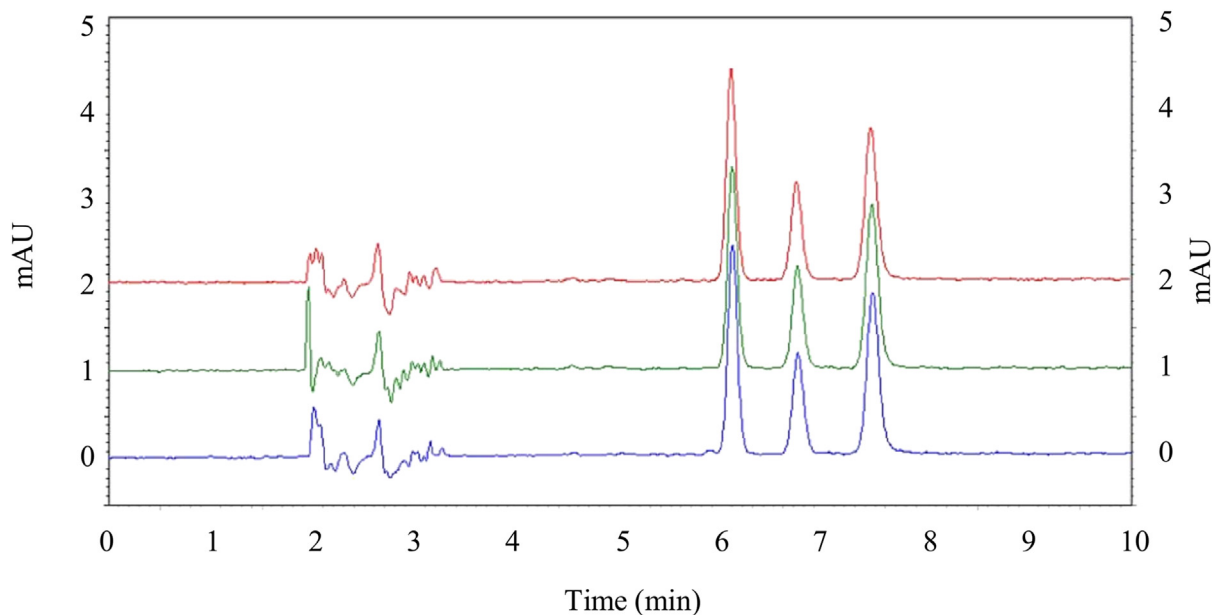
## 3. Results and Discussion

This study was carried out at a low concentration of standard curcuminoids as mentioned in the Thai Herbal Pharmacopoeia that used UV-visible (UV-vis) spectrophotometry. UV-vis spectrophotometry can investigate only the total concentration of curcuminoids, while HPLC can determine both individual and total curcuminoids. Thus, HPLC was selected in this work. Previously, determination of curcuminoid contents using a simple isocratic HPLC was reported [11]. The mobile phase system consisted of 2%v/v acetic acid and acetonitrile (60:40) at a flow rate of 2.0 mL/min, the curcuminoids were eluted at retention time of 10.8 minutes, 12.1 minutes, and

13.6 minutes for bisdemethoxycurcumin, demethoxycurcumin, and curcumin, respectively. The other publications had reported an isocratic mobile phase system consisting of acetonitrile/methanol/water (40:20:40, v/v) with a flow rate of 1 mL/min. The results showed that three curcuminoids were eluted at retention times of 8.8 minutes, 9.5 minutes, and 10.2 minutes [12]. Furthermore, other studies showed curcuminoids that were separated between 10 minutes and 15 minutes [13] or > 15 minutes [14]. However, our work successfully developed a rapid HPLC condition that took less time to elute curcuminoids bisdemethoxycurcumin, demethoxycurcumin, and curcumin (retention times of 6.0 minutes, 6.7 minutes, and 7.4 minutes, respectively; Figure 2).

Linear equation, range,  $R^2$ , LOD, and LOQ of individual curcuminoids are shown in Table 1. For precision, %RSD of bisdemethoxycurcumin, demethoxycurcumin, and curcumin at three concentration levels was less than 2% and 5% for intraday and interday precision, respectively. For accuracy, the percentage recovery was 99.96–101.14%, 97.42–102.23%, and 98.01–99.12% for bisdemethoxycurcumin, demethoxycurcumin, and curcumin, respectively. These results indicated that this HPLC system was precise and accurate. Precision and accuracy results of three curcuminoids are shown in Table 2. System suitability results showed that % RSD of all parameters were < 2%. Besides, the theoretical plate of three curcuminoids were > 2000, indicating that this system was suitable (Table 3).

Determination of individual curcuminoid contents in turmeric capsules for all nine lots from three hospitals showed higher contents of curcumin than bisdemethoxycurcumin and demethoxycurcumin, and that the content of bisdemethoxycurcumin and demethoxycurcumin were similar. Content of curcumin, demethoxycurcumin, and



**Figure 2** – High performance liquid chromatography chromatogram of curcuminoids from turmeric capsules produced in Hospital A dissolved in dissolution medium for 60 minutes. The blue, green, and red chromatogram indicated lot 1, 2, and 3, respectively. Compounds eluted at retention times of 6.0 minutes, 6.7 minutes, and 7.4 minutes were bisdemethoxycurcumin, demethoxycurcumin, and curcumin, respectively.

**Table 1 – Linearity, limit of detection (LOD), and limit of quantitation (LOQ) of individual curcuminoids.**

| Parameters                      | Bisdemethoxycurcumin    | Demethoxycurcumin       | Curcumin               |
|---------------------------------|-------------------------|-------------------------|------------------------|
| Linear equation                 | $Y = 171188 X - 2136.4$ | $Y = 160600 X - 3623.8$ | $Y = 164830 X - 18527$ |
| Test range ( $\mu\text{g/mL}$ ) | 0.019–0.600             | 0.053–3.400             | 0.250–8.000            |
| $R^2$                           | 0.9995                  | 0.9999                  | 0.9997                 |
| LOD ( $\text{ng/mL}$ )          | 2.25                    | 1.7                     | 3                      |
| LOQ ( $\text{ng/mL}$ )          | 11.25                   | 12.75                   | 6                      |

**Table 2 – Result of precision and accuracy studies ( $n = 3$ ).**

| Compounds            | Concentration ( $\mu\text{g/mL}$ ) | Precision (%RSD) |          | Added amount ( $\mu\text{g/mL}$ ) | Accuracy Recovery (%) |
|----------------------|------------------------------------|------------------|----------|-----------------------------------|-----------------------|
|                      |                                    | Intraday         | Interday |                                   |                       |
| Bisdemethoxycurcumin | 0.075                              | 1.11             | 1.56     | 0.075                             | 101.14 $\pm$ 1.72     |
|                      | 0.150                              | 0.81             | 3.22     | 0.150                             | 99.96 $\pm$ 1.31      |
|                      | 0.300                              | 0.51             | 2.20     | 0.300                             | 100.79 $\pm$ 0.08     |
| Demethoxycurcumin    | 0.106                              | 0.86             | 3.14     | 0.106                             | 100.73 $\pm$ 0.81     |
|                      | 0.213                              | 1.61             | 2.59     | 0.213                             | 102.23 $\pm$ 0.77     |
|                      | 0.425                              | 1.67             | 3.62     | 0.425                             | 97.42 $\pm$ 0.15      |
| Curcumin             | 0.500                              | 1.40             | 2.71     | 0.500                             | 98.01 $\pm$ 0.65      |
|                      | 1.000                              | 0.64             | 1.40     | 1.000                             | 98.51 $\pm$ 1.41      |
|                      | 2.000                              | 0.61             | 2.73     | 2.000                             | 99.12 $\pm$ 0.63      |

**Table 3 – System suitability results ( $n = 6$ ).**

| Compounds                                      | Retention time    | Peak area         | Theoretical plates (USP) | Asymmetry       |
|------------------------------------------------|-------------------|-------------------|--------------------------|-----------------|
| Bisdemethoxycurcumin (0.038 $\mu\text{g/mL}$ ) |                   |                   |                          |                 |
| Mean $\pm$ SD                                  | 6.026 $\pm$ 0.005 | 4181 $\pm$ 51     | 19719 $\pm$ 336          | 1.07 $\pm$ 0.02 |
| %RSD                                           | 0.08              | 1.22              | 1.70                     | 1.80            |
| Demethoxycurcumin (0.213 $\mu\text{g/mL}$ )    |                   |                   |                          |                 |
| Mean $\pm$ SD                                  | 6.667 $\pm$ 0.004 | 30326 $\pm$ 499   | 17043 $\pm$ 137          | 1.05 $\pm$ 0.02 |
| %RSD                                           | 0.06              | 1.65              | 0.80                     | 1.40            |
| Curcumin (1.000 $\mu\text{g/mL}$ )             |                   |                   |                          |                 |
| Mean $\pm$ SD                                  | 7.400 $\pm$ 0.004 | 144919 $\pm$ 1211 | 16308 $\pm$ 93           | 1.16 $\pm$ 0.02 |
| %RSD                                           | 0.06              | 0.84              | 0.57                     | 1.58            |

RSD = relative standard deviation; SD = standard deviation.

bisdemethoxycurcumin of nine lots of turmeric capsules were 6.61–7.67%w/w (average, 7.21%w/w), 2.76–3.33%w/w (average, 3.04%w/w), and 2.64–3.47%w/w (average, 3.10%w/w), respectively. Moreover, the total curcuminoid contents ranged from 12.02%w/w to 14.36%w/w with an average value of 13.35%w/w. Both intrinsic and extrinsic factors affected

curcuminoid contents including seasonal variation, environmental condition, postharvested handling, storage, manufacturing, microbial contamination, etc. [15]. Thus, the variation of curcuminoid contents in turmeric capsules was observed in this work. The Thai Herbal Pharmacopoeia (1995) [10] and the 2013 National List of Essential Medicines

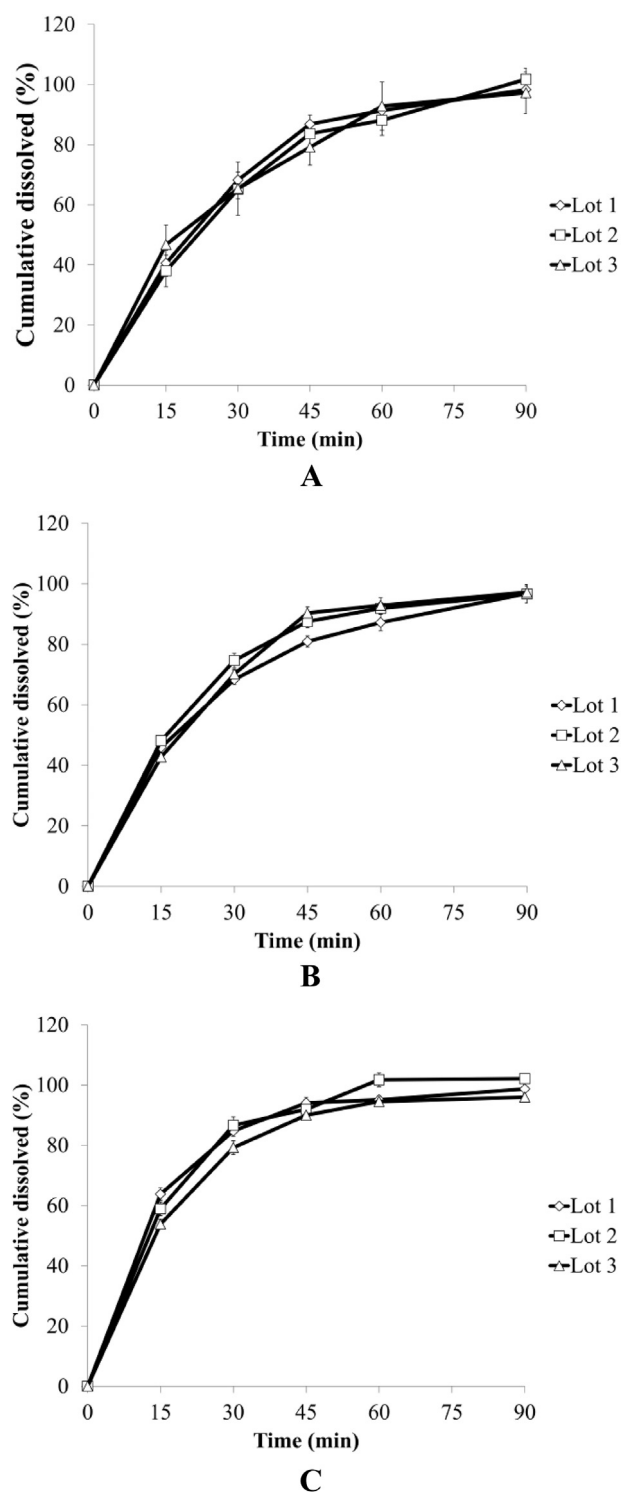
**Table 4 – Individual curcuminoids, total curcuminoids, and volatile oil contents.**

| Hospital | Lot | Bisdemethoxycurcumin (%w/w) | Demethoxycurcumin (%w/w) | Curcumin (%w/w) | Total curcuminoid contents (%w/w) | Volatile oil contents (%v/w) |
|----------|-----|-----------------------------|--------------------------|-----------------|-----------------------------------|------------------------------|
| A        | 1   | 3.08 $\pm$ 0.06             | 3.10 $\pm$ 0.02          | 7.35 $\pm$ 0.04 | 13.53 $\pm$ 0.06                  | 8.00 $\pm$ 0.00              |
|          | 2   | 3.17 $\pm$ 0.03             | 3.06 $\pm$ 0.02          | 7.22 $\pm$ 0.03 | 13.45 $\pm$ 0.02                  | 8.00 $\pm$ 0.00              |
|          | 3   | 3.36 $\pm$ 0.01             | 3.33 $\pm$ 0.02          | 7.67 $\pm$ 0.03 | 14.36 $\pm$ 0.05                  | 8.00 $\pm$ 0.00              |
| B        | 1   | 3.02 $\pm$ 0.04             | 3.06 $\pm$ 0.04          | 7.30 $\pm$ 0.06 | 13.39 $\pm$ 0.14                  | 7.00 $\pm$ 0.00              |
|          | 2   | 2.64 $\pm$ 0.01             | 2.76 $\pm$ 0.02          | 6.61 $\pm$ 0.01 | 12.02 $\pm$ 0.02                  | 7.25 $\pm$ 0.35              |
|          | 3   | 2.97 $\pm$ 0.02             | 2.96 $\pm$ 0.03          | 7.07 $\pm$ 0.03 | 13.00 $\pm$ 0.07                  | 8.00 $\pm$ 0.00              |
| C        | 1   | 2.87 $\pm$ 0.01             | 2.83 $\pm$ 0.03          | 6.90 $\pm$ 0.01 | 12.60 $\pm$ 0.04                  | 7.00 $\pm$ 0.00              |
|          | 2   | 3.36 $\pm$ 0.05             | 3.14 $\pm$ 0.02          | 7.37 $\pm$ 0.06 | 13.88 $\pm$ 0.07                  | 7.00 $\pm$ 0.00              |
|          | 3   | 3.47 $\pm$ 0.05             | 3.08 $\pm$ 0.05          | 7.39 $\pm$ 0.01 | 13.94 $\pm$ 0.05                  | 7.00 $\pm$ 0.00              |
| Average  |     | 3.10 $\pm$ 0.27             | 3.04 $\pm$ 0.17          | 7.21 $\pm$ 0.31 | 13.35 $\pm$ 0.72                  | 7.47 $\pm$ 0.51              |

The contents were reported as mean  $\pm$  standard deviation ( $n = 3$ ).



specified total curcuminoid contents in turmeric capsules should be higher than 5%w/w [7]. This work found that total curcuminoid contents of all lots of turmeric capsules met the above criteria. The individual and total curcuminoid contents are shown in Table 4.



**Figure 3** – Dissolution profile of total curcuminoids in turmeric capsules produced in: (A) Hospital A; (B) Hospital B; and (C) Hospital C (n = 6).

Dissolution profiles of total curcuminoids in turmeric capsules from three hospitals are shown in Figure 3. Dissolution of total curcuminoids showed that curcuminoids solubilize well in dissolution medium, approximately 40–60% was dissolved within the first 15 minutes. During the 15–90 minutes of the dissolution test, dissolution profiles were fitted with a Higuchi model. Dissolution profiles of curcuminoids were similar for all nine lots of turmeric capsules. The Thai Herbal Pharmacopoeia (Supplement 2011) specified that cumulative amount of total curcuminoids dissolved in 60 minutes should not be less than 75% [9]. Results showed that cumulative amount of total curcuminoids dissolved of all nine lots was higher than 80% in 60 minutes, indicating that all nine lots of turmeric capsules met the criteria. However, curcuminoids had poor water solubility and relatively poor bioavailability, thus, solubility enhancement should be of concern during formulation development [16].

Results of the volatile oil content of all nine lots of turmeric capsules are shown in Table 4. The Thai Herbal Pharmacopoeia (1995) specified that volatile oil content should not be less than 6%v/w [10]. The volatile oil content of all nine lots of turmeric capsules ranged from 7.00%v/w to 8.00%v/w, indicating that the volatile oil content of the nine lots of turmeric capsules met the criteria.

We successfully validated a simple and rapid reversed phase-HPLC for the determination of individual and total curcuminoids in turmeric capsules. Total curcuminoid contents of all lots of turmeric capsules were higher than 5%w/w. However, the method for determination of total curcuminoid contents was dissimilar from the Thai Herbal Pharmacopoeia as this study used HPLC instead of UV-vis spectrophotometry. Furthermore, dissolution profiles of curcuminoids were fitted with Higuchi model. In addition, the volatile oil content of all lots was higher than 6%v/w. In summary, all nine lots of turmeric capsules from three hospitals met the standard criteria of the Thai Herbal Pharmacopoeia in the topic of curcuminoid contents, dissolution, and volatile oil content.

## Conflicts of interest

All authors declare no conflicts of interest.

## Acknowledgments

The authors would like to thank for the Faculty of Pharmacy, Rangsit University for facilities as well as Sun Herb Thai Chinese Manufacturing Plant for supporting some chemicals. This study was funded by the Research Institute of Rangsit University (Grant no. 8/2557).

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