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Research Article

Simple quantitative analysis of α-mangostin in mangosteen rind extracts and their microparticle preparations using HPLC method

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Abstract: Method validation is a crucial process of quantitative analysis of active constituents to standardize medicinal herbs and their products. *Garcinia mangostana* or mangosteen has become a fruit of choice in various types of industries and therefore, standardization of this plant is of paramount. In this study, the HPLC validation method of α-mangostin has been proposed and validated for linearity, accuracy, precision, limit of detection (LOD) and limit of quantitation (LOQ). The method was linear from the α-mangostin concentration ranges of 5-300 µg/ml with the correlation coefficient of 0.9997. The percent RSD, indicative of precision, was lower than 2%, which demonstrate the precision of the system. The average percent recovery was 100.04 ± 0.63%. The LOD and LOQ were 0.07 and 0.20 µg/ml, respectively. The validated method was then used to quantitatively analyze the content of α-mangostin in extracts and their respective microparticle preparations. Alpha-mangostin contents were 49.60 ± 0.21, 14.13 ± 0.07, and 13.17 ± 0.01 %w/w for dichloromethane, 95% ethanol and 50% ethanol extracts while the contents of α-mangostin in 100 g of microparticle preparations containing dichloromethane, 95% ethanol and 50% ethanol extracts were 118.49 ± 0.98, 45.13 ± 0.23 and 38.70 ± 0.15 mg, respectively.

Keywords: HPLC, mangosteen, α-mangostin, microparticle, method validation.

INTRODUCTION

Mangosteen (*Garcinia mangostana* Linn.), of the family Guttiferae, is an important economical fruit in Southeast Asian countries. Regarded as "the queen of fruits", the white and juicy aril part is popularly consumed while the dark purple rind is usually discarded. However, the rind has proven to be reasonably nutritious as it contains many compounds, including flavonoids, xanthones and tannins; and therefore possessing many biological activities[1, 2]. Ethnologically, mangosteen rind has been used to treat an array of diseases, for instance, abdominal pain, diarrhea, dysentery, skin infection, suppuration and chronic ulcer. Currently, the rind of mangosteen has been utilized in cosmetic formulation due to its anti-acne activity and in drinks and food because of its potent anti-oxidant activity[3, 4].

Since the rind has been extensively utilized in the industry, a standard method that can accurately and quantitatively determine the amount of the mangosteen's active compound is essential. Due to its wide range of healing aptitude, it is expected that mangosteen rind contains many active constituents. However, one constituent, α-mangostin, is a major and active compound with potent anti-bacterial, antioxidant and anti-inflammatory properties[5, 6]. Therefore, it is convenient to perform a routine quality control of crude extract or product using this compound as a marker.

Hence, in this study, we aim to quantitatively analyze α-mangostin in mangosteen crude extracts and its microparticle products using HPLC. Although the method to do so has been proposed already, this method employs methanol, a less toxic solvent than acetonitrile, and acetic acid, which does not precipitate in the presence of organic solvent[7]. This method also holds its advantage of a simple and faster linear gradient elution system and yields a higher resolution than the proposed method[8].

EXPERIMENTALS

Chemicals and reagents: Alpha-mangostin was purchased from Chroma Dex Inc. (Santa Ana, CA). HPLC grade methanol was purchased from Fisher (USA). Acetic acid was of analytical grade and was purchased from Lab Scan (Thailand). Commercial grade dichloromethane was purchased from TTK Science (Thailand). Commercial grade ethanol was purchased from Excise Department, Bangkok, Thailand. Commercial grade solvents were distilled before use.

Plant materials: The ripe fruits of *G. mangostana* were purchased from a local market in Bangkok, Thailand in May 2013. The specimens, identified by Dr. Wandee Gritsanapan, were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University. After the edible part was removed, the rinds were cleaned with tap water, cut into small pieces, and dried in a hot air oven at 60°C for 40 h. The dried sample was roughly ground and kept in a tight container protected from light.

Preparation of standard solutions: The reference standard of α-mangostin was prepared by dissolving accurately weighed 100 mg of α-mangostin in 100 ml of methanol in a volumetric flask. Various concentrations of standard solutions were diluted to obtain final concentrations at 300, 100, 50, 25, 10 and 5 µg/ml with methanol.

Extraction of the rind and preparation of sample solutions: The ground mangosteen rind (800 g) was separately put into a thimble and extracted with 2.5 L of dichloromethane, 95% ethanol, and 50% ethanol in a Soxhlet apparatus for 40 h. Each extract was then filtered and concentrated under reduced pressure at 40°C. Each sample was done in triplicate.

Each dried extract of mangosteen rind was accurately weighed and dissolved in methanol to obtain the concentration of 1 mg/ml. It was then diluted to 100 µg/ml. The sample was filtered through 0.2 µm

nylon membrane (Lubitech, China) and subjected to validated HPLC method to determine α-mangostin content.

Preparation of micro particle containing mangosteen extract: Microparticle was prepared by mixing sodium alginate solution, polyvinyl alcohol solution and each mangosteen extract dissolved in ethanol, and stirring until the mixture became homogenized. The mixture was loaded into a syringe and pushed through a needle into a stirring calcium chloride solution. After the wall of the microparticle beads hardened, they were washed with and stored in distilled water[9].

HPLC and chromatographic conditions: HPLC method validation and analysis were performed on a Shimadzu SPD-10A (Japan) equipped with LC-10AD pump, DGU-10A degasser, UV-vis detector SPD-10AV and auto-injector SIL-10AD. Hypersil BDS C-18 column (4.6 x 150 mm i.d. 5 µm) with a C-18 guard column was used. Mobile phase A was 0.1% acetic acid in water and B was methanol. Linear gradient elution of 82% B was used for 15 min, 100% B for 10 min, and the column was reequilibrated with 82% B for 10 min. The flow rate was 1 ml/min at ambient temperature. The injection volume was 10 µl and the detector was set at 254 nm.

Method validation: Validation of the analytical method was performed in accordance to the International Conference on Harmonization guideline[10]. The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity: Linearity was determined by analyzing six concentrations (300, 100, 50, 25, 10, and 5 µg/ml) α-mangostin standard solutions in triplicate. The calibration curve was obtained by plotting the peak areas against respective concentrations of standards.

Precision: Intraday precision was determined by analyzing 50, 100, and 200 µg/ml solution of α-mangostin seven times within one day and the interday precision was analyzed for 3 consecutive days by the proposed method.

Accuracy: Standard addition was performed with three different concentrations of standard solutions. Spike samples were prepared in triplicate and three different determinations were done.

*LOD and LOQ:*Determination of signal-to-noise ratio was calculated under the chromatographic condition. LOD was considered as 3:1 and LOQ as 10:1. LOD and LOQ were confirmed by injecting the calculated amount using the method.

Statistical analysis and calculation: Statistical analysis was done using SPSS. Resolution was calculated using equation:

$$R_s = \frac{(t_{R1} - t_{R2})}{0.5(t_{w1} - t_{w2})}$$

where t_R is retention time and t_w is width at the baseline of the peak.

Tailing factor was calculated using equation:

$$T = \frac{(a + b)}{2a}$$

where a is distance from the leading edge of the peak to the midpoint and b is the distance from the point at the peak midpoint to the trailing edge.

RESULTS AND DISCUSSION

Standardization of herbal medicine is an essential step for quality establishment of its products. Preferably, a marker compound used for standardization has to be identified and it should be a major or active compound, or both. In this study, since α-mangostin is both major and active compound, it was chosen as a marker compound for this method validation process. The proposed method proved to be linear, accurate and precise with excellent resolution and acceptable tailing factor as shown in Table 1.

Table 1: Validation parameters, linear range, LOD and LOQ; and resolution and tailing factor.

Parameters	Results
Linear range (µg/ml)	5-300 µg/ml
Regression equation	y = 41825x - 121324
Correlation Coefficient (r^2)	0.9997
LOD (µg/ml)	0.07
LOQ (µg/ml)	0.20
Resolution to the nearest peak	6.7
Tailing factor	1.08

The validated method yields a linear calibration curve within the concentration ranging from 5 – 300 µg/ml with the correlation coefficient (r^2) of 0.9997 (Figure 1). The method also displayed acceptable precision, with the RSD values lower than 2%. The recovery at three different concentrations, 99.21 ± 1.49, 100.72 ± 0.29 and 100.21 ± 0.52, indicated the acceptable accuracy of the method. LOD and LOQ were found to be 0.07 and 0.20 µg/ml, respectively. The validated parameters were shown in Tables 2 and 3.
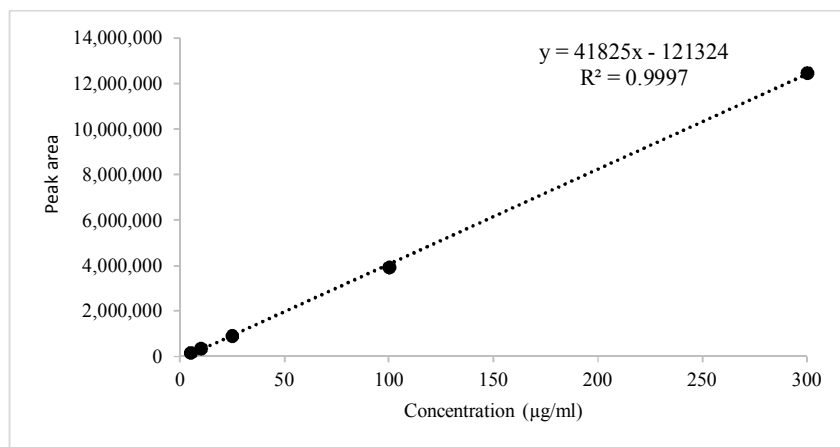

Figure 1: Calibration curve of α-mangostin.

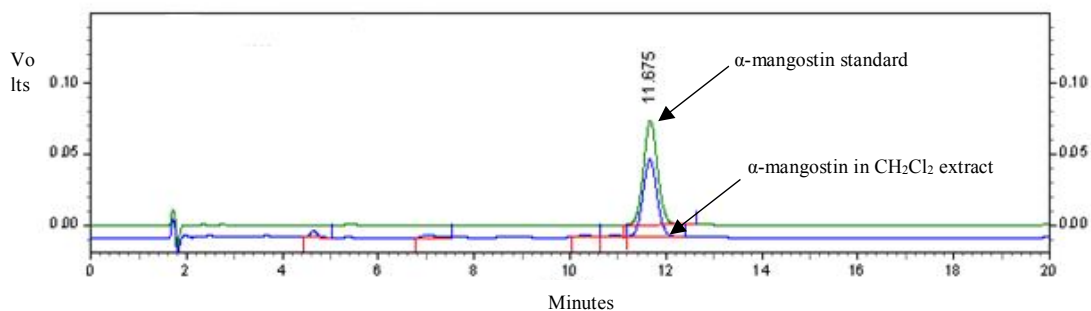**Table 2:** Validation parameter - precision

Concentration (µg/ml)	Intraday% RSD			Interday %RSD
	Day 1	Day 2	Day 3	
50	0.018	0.232	0.289	0.855
100	0.010	0.504	0.277	0.816
200	0.292	0.334	0.493	0.827

Table 3: Validation parameter - accuracy

Number	Recovery (%)
1	99.21 ± 1.49
2	100.72 ± 0.29
3	100.21 ± 0.52
Average	100.05 ± 0.63

The validated method was then used to quantitatively analyze the content of α-mangostin in three different types of extracts and their respective microparticle products. HPLC chromatogram of dichloromethane extract and α-mangostin standard is shown in Figure 2. The results are shown in Table 4 and Table 5.

Figure 2: HPLC chromatograms of α-mangostin and dichloromethane extract, with α-mangostin's retention time at 11.6 min.

Table 4: Amount of α-mangostin in three different types of extracts.

Samples	Amount of α-mangostin present in extract (% w/w)
CH_2Cl_2 extract	49.60 ± 0.21
95% EtOH extract	14.13 ± 0.07
50% extract	13.17 ± 0.01

Table 5: Amount of α-mangostin in three 100 g of microparticle preparations

Samples	Amount of α-mangostin found (mg)
Microparticle of CH_2Cl_2 extract	118.49 ± 0.98
Microparticle of 95% EtOH extract	45.13 ± 0.23
Microparticle of 50% EtOH extract	38.70 ± 0.15

It was found that the amount of α-mangostin were 49.60 ± 0.21, 14.13 ± 0.07 and 13.17 ± 0.01 for dichloromethane, 95% ethanol, and 50% ethanol extracts, respectively and dichloromethane extract contained significantly the highest amount of α-mangostin ($p < 0.05$). The amount of α-mangostin in 100 g of microparticle preparations were 118.49 ± 0.98, 45.13 ± 0.23 and 38.70 ± 0.15 mg for dichloromethane, 95% ethanol and 50% ethanol extracts microparticle preparation , respectively.

Considering the parameters that were validated, the proposed method has proven to be precise, accurate, and competently sensitive for the qualitative analysis in laboratory scale and could potentially be applied in the industrial scale. As shown, the method could be employed to determine the amount of α-mangostin in both mangosteen extracts and microparticle products. This method is also more applicable in the industrial scale, where factors such as disposal of toxic solvents and cost have exponential effects compared to smaller laboratory scale, because cheaper and less hazardous solvent, methanol, was used instead of acetonitrile. Acetic acid was also used instead of phosphoric acid and it is less likely to precipitate and clog the column, lengthening column life time. This system is also faster, therefore it is more efficient when a large number of samples were to be analyzed.

CONCLUSION

The proposed method was found to be linear within the aforementioned concentration range, precise and accurate. The method was able to quantitatively analyze the concentration of α-mangostin in extracts and microparticle preparations. It also yielded a good resolution and employed less toxic and less expensive solvents than the previously reported methods, making it more applicable in a larger industrial scale.

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