Validation of LC for the Determination of α-Mangostin in Mangosteen Peel Extract: A Tool for Quality Assessment of *Garcinia mangostana* L.

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Abstract

Mangosteen, *Garcinia mangostana* L., is known as the "Queen of fruits" and can be cultivated in the tropical rainforest such as Malaysia, Indonesia, and Thailand. Compounds isolated from the fruit peel of mangosteen contain abundant xanthones (especially α-mangostin). It has been used as traditional medicine such as anti-inflammatory and antibacterial and is popularly applied to cosmetic and pharmaceutical products. However, there is little information for quality and quantity determination of α-mangostin in mangosteen. Thus, the aim of this study was to set up a validated and stability-indicated isocratic reverse-phase high-performance liquid chromatographic (HPLC) method for quality control and quantity determination of α-mangostin from mangosteen peel extract. The assay was fully validated and shown to be linear (r^2 > 0.999), sensitive (LOD = 0.02 µg/mL and LOQ = 0.08 µg/mL), accurate (intra-day was between 98.1–100.8%, inter-day was between 90.0–101.3%), precise (intra-day variation ≤ 1.8%, inter-day variation ≤ 4.3%), specific, and with good recovery. Total analysis was ~ 8 min. The finalized method is also a stability-indicating assay. The present method should be useful for analytical research and for routine quality control analysis of α-mangostin in mangosteen peel extract and products of mangosteen.

Introduction

Mangosteen, *Garcinia mangostana* L., is known as the "Queen of fruits" in Asia and can be cultivated in the tropical rainforest such as Malaysia, Indonesia, and Thailand (1). Compounds isolated from the fruit peel of mangosteen contain abundant xanthones, for example: α-mangostin, β-mangostin, γ-mangostin, gartanin, 8-deoxygartanin, and mangostanol (2–5). α-Mangostin (Figure 1) is the major component, and it has been used worldwide as traditional medicine for anti-inflammatory (6,7), antibacterial (8-10), and anticancer effects (11,12). Nowadays, it is popularly applied to cosmetic and pharmaceutical products. Because α-mangostin represents the majority of the clinical benefits of this herbal medicine, it is reasonable and logical to determine the concentration of α-mangostin as a chemical marker for the quality control of *G. mangostana* and its products, which usually is the only xanthone ingredient quantity-marked in label. Recently, our research has concerned the evaluation of products of mangosteen, such as antibacterial film prepared from mangosteen peel extract. However, there is little information for quality and quantity determination of α-mangostin in mangosteen. There is a recent report using high-pressure liquid chromatography with photodiode array detector (HPLC-PDA) at 320 nm to detect and quantify α-mangostin and five other xanthones from *G. mangostana* (13). However, because only the minor of the five xanthones in products are reported and it has a very long analysis time, the method does not permit application for routine analyses. Furthermore, it has a higher maximum absorbance of α-mangostin at 240 nm; therefore, the aim of this study is to set up a reverse-phase HPLC–UV method at 240 nm for quality control and quantity determination of α-mangostin from mangosteen peel extract, with the total run time of the method per sample at just 8 min, which is also shorter than the reported one. Thus the speed of analysis is suitable for routine measurement of α-mangostin in not only any product preparations, but also in crude extract of *G. mangostana*. This method was fully validated according to International Conference on Harmonization (ICH) of note for guidance on validation of

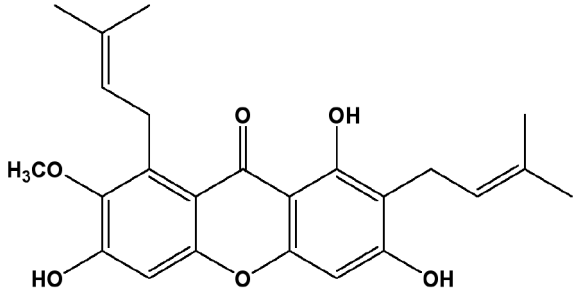

Figure 1. Chemical structure of α-mangostin.

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analytical procedures (14), which is the first report that would serve as a stability-indicating assay method for α-mangostin in the presence of degradation products. Furthermore, the sensitivity was evaluated for its application; it was expected that this method would be efficient in analyzing low concentrations of α-mangostin in antibacterial film prepared from mangosteen peel extract.

Experimental

Chemicals and reagents

Standard α-mangostin was purchased from Chromadex Inc. (Santa Ana, CA; purity of 96.5%). Acetonitrile (HPLC grade) and methanol (AR grade) were purchased from Labscan Asia Co., Ltd. (Bangkok, Thailand), and formic acid was obtained from May & Baker Ltd. (Dagenham, England). The water was purified using a Milli-Q system (Milford, MA).

Instrumentation and chromatographic condition

The HPLC system (Shimadzu, Kyoto, Japan) consisted of a quaternary solvent delivery system (LC-10ADvp), autosampler (SIL-10ADvp), solvent degasser (DGU-14A), and UV detector (SPD-10ADvp). The UV spectra were recorded in the 200–400 nm range, with a PDA (Agilent 1100 HPLC system), and the quantification wavelength was set at 240 nm.

Chromatographic separation was carried out at room temperature using a Hypersil BDS C_{18} analytical column (125 × 4.0 mm i.d., 5 µm) with C_{18} guard column. The isocratic mobile phase consisted of 0.2% formic acid–acetonitrile (30:70, v/v), which was pumped at a flow rate of 1.0 mL/min. The injection volume was 20 µL.

Preparation of plant materials

The fruit peels of mangosteen were collected from Chumporn province (south of Thailand). The fruit peels were dried at 50°C, powdered, and extracted by dichlormethane. The ground, dried peels of plant were then concentrated to dryness under reduced pressure. The sample was prepared by accurately weighing 10 mg of mangosteen extract into a 100-mL volumetric flask. Approximately 60 mL of methanol was added, and the solution was sonicated for 15 min. The solution was allowed to cool to room temperature before being filled up to the final volume of 100.0 mL. After centrifugation for ~ 10 min, 10 mL of the supernatant was diluted to 100 mL, in a volumetric flask by acetonitrile and filtered through a 0.45-µm filter membrane before analysis. Twenty microliters of the sample solution was directly injected into the HPLC column and separated under described chromatographic conditions.

Preparation of standards and calibration standard solution

The standard stock solutions of α-mangostin were prepared by dissolving their accurately weighted compounds in methanol to give the solution a final concentration of 100 µg/mL, and stored at 4°C until use. These solutions were then serially diluted with methanol to provide calibration standard solutions of 1.0, 5.0, 10.0, 15.0, and 20.0 µg/mL.

Stability-indicating assay

Forced degradation of α-mangostin was carried out under thermolytic, photolytic, acid/base hydrolytic, and oxidative stress conditions. Thermal (in a controlled-temperature oven at 80°C for 3 h) and photo-degradation (under UV radiation having peak intensities at 254 and 366 nm for 6 h) were preceded in solid state. After degradation, stock solutions were prepared by dissolving in methanol to achieve a concentration of 500 µg/mL. From these solutions, aliquots were diluted with 50% methanol to achieve a concentration of 25 µg/mL.

For hydrolytic and oxidative degradation, solutions were prepared by dissolving α-mangostin in extract in a small volume of methanol, and later dropped with 3% hydrogen peroxide (3% H_2O_2), 3N HCl, or 3N NaOH solution and heated at 80°C for 3 h. After degradation, the stock solution was prepared by dissolving in methanol to achieve concentration of 500 µg/mL. From these solutions, aliquots were diluted with 50% methanol to achieve a concentration of 25 µg/mL. All the sample solutions for acid/base hydrolysis and oxidative stress were kept in a dark to prevent the effect of light.

Assay characteristics for method validation

Specificity

The specificity was determined by analysis of a solution containing 10 µg/mL of α-mangostin both for standard and mangosteen peel extract solution. Methanol was used as a control. A volume of 20 µL was individually injected into the HPLC system previously described. The specificity was then performed by comparing the retention times of α-mangostin in the chromatogram of the extract solution with those in the chromatogram of the standard solution.

Linearity and calibration curve

Standard α-mangostin solutions in the 1.0, 5.0, 10.0, 15.0, and 20.0 µg/mL range were injected into the HPLC system. Six replicate analyses were performed per day. The calibration curve was analzed using the linear least-squares regression equation. Calibration curves were constructed by plotting peak area against the concentration of standards. A correlation coefficient above 0.99 was acceptable.

Accuracy and precision

Intra- and inter-day precision and accuracy were evaluated at three different levels of standard α-mangostin concentrations (5.0, 10.0, and 15.0 µg/mL). Intra- and inter-day assay precision were determined as relative standard deviation (RSD), and intra- and inter-day assay accuracies were expressed as percentages of theoretical concentration, as accuracy (%) = (found concentration / theoretical concentration) × 100. Intra-day assay involved three replicates per day and inter-day assay were performed on three separate days.

Recovery

Three level differences of standard α-mangostin concentrations were spiked in dried mangosteen peel extract sample with known contents of α-mangostin, and the samples were processed according to the "Preparation of plant materials" procedure making the final concentration of standards to be as 5.0,

10.0, and 15.0 µg/mL. The three injections for each concentration were done per day over three different days. The recoveries of α-mangostin were calculated as the following equation:

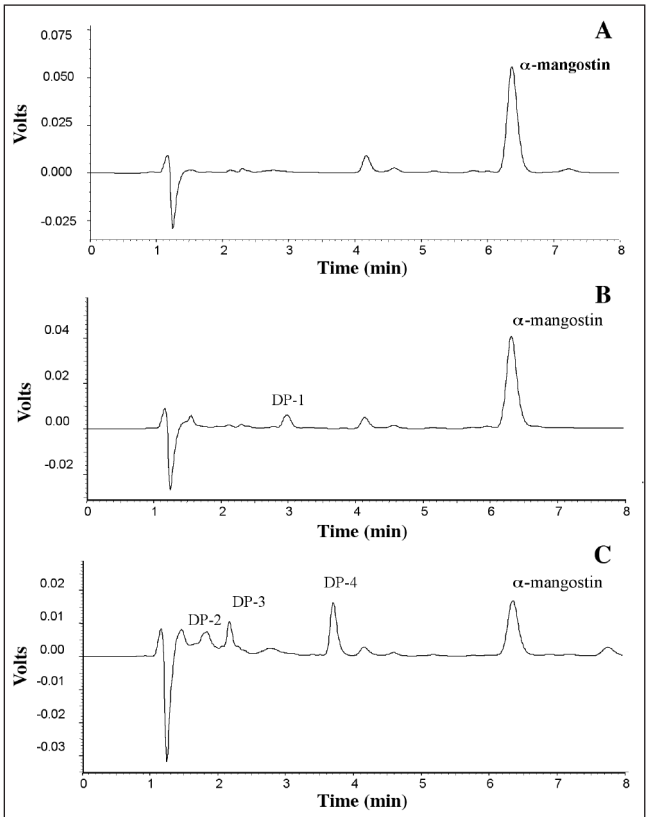

Figure 2. Typical HPLC chromatograms of α-mangostin (15 µg/mL) under stress condition untreated extract solution (A), 3% H_2O_2 (B), and 3N HCl (C). Key: DP-1, degradation product formed in H_2O_2; DP-2, DP-3, and DP-4, formed in HCl.

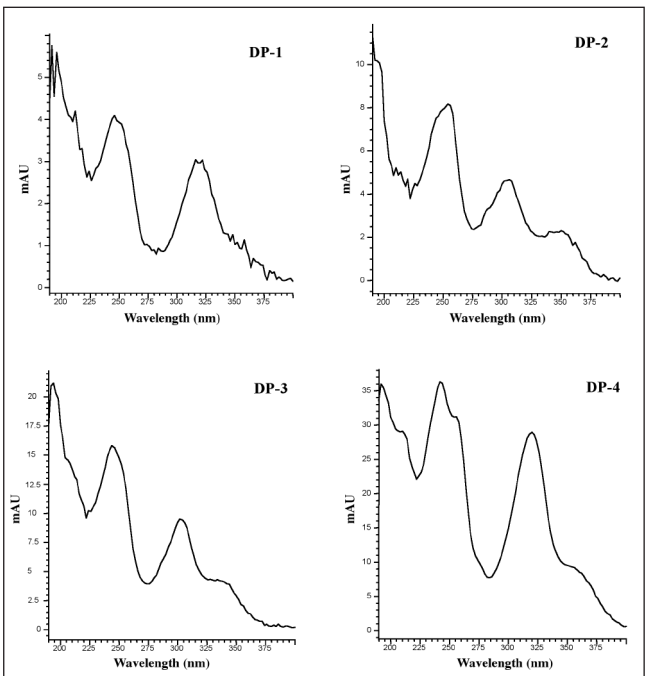

Figure 3. UV spectra of degradation products (DP) 1–4 by a PDA detector (corresponding to peaks in Figure 2B and 2C).

$$\text{Recover (\%)} = \frac{C_{obs} - C_{blk}}{C_{act}} \times 100$$

where: C_{obs} is the observed concentration of α-mangostin detected in the sample solution (µg/mL). C_{blk} is the concentration of α-mangostin detected in mangosteen peel extract sample solution without added standard α-mangostin solution (µg/mL). C_{act} is the actual concentrations of standard α-mangostin solution (µg/mL).

Limits of detection and quantitation

For the evaluation of the limits of detection (LOD) and quantitation (LOQ), a concentration sequence of the standards was prepared by diluting standard solutions with methanol and was then analyzed with the HPLC system. LOD and LOQ were based on three times and ten times of signal-to-noise ratio, respectively.

Results and Discussion

To develop a precise, accurate, specific, sensitive, and suitable stability-indicating HPLC method for assay of α-mangostin, the proposed chromatographic condition was found appropriate for quantitative determination in the presence of degradation products and impurities.

Stability-indicating assay

HPLC studies under different stress conditions indicated the following degradation behaviors. It was found that α-mangostin was stable under light, heat, and basic hydrolytic under conditions used. Nevertheless, the α-mangostin demonstrated decomposition in acidic hydrolytic and oxidative conditions, but the degradation products (DP) have no interference with this analytical method, as shown in Figure 2. Figure 3 shows the shape of UV absorption spectra of degradation products 1–4 by a PDA detector (corresponding to their peaks in Figure 2).

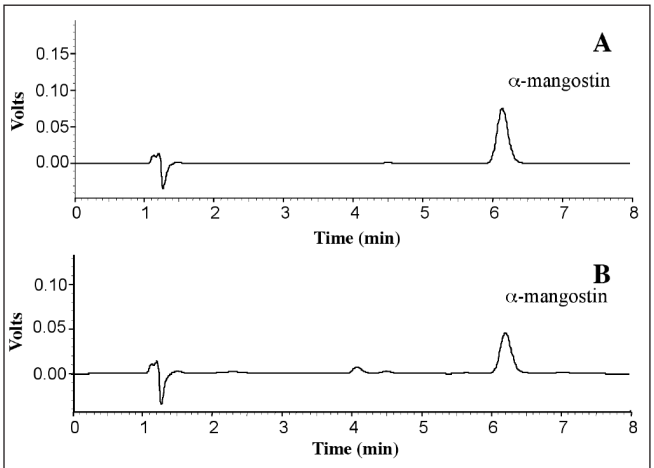

Figure 4. HPLC chromatograms showing α-mangostin standard solution (10 µg/mL) (A), mangosteen peel extract sample solution (contained α-mangostin 10 µg/mL) (B).

Validation of the developed stability-indicating method

Specificity

The representative HPLC chromatograms obtained from standard α-mangostin and mangosteen peel extract solution are shown in Figure 4. It shows that no other co-eluting peak was found that would interfere with the main peaks of α-mangostin, suggesting satisfactory specificity of the method with the retention time of 6.2 min. The retention time is consistent with RSD lower than 0.2% (n = 3, data not shown). Furthermore, there were also no co-eluting peaks from any of the stress condition; thus, we determined this method to be very specific for α-mangostin. The shape of photodiode array spectra of α-mangostin in standard was the same pattern as from mangosteen peel extract solution (Figure 5).

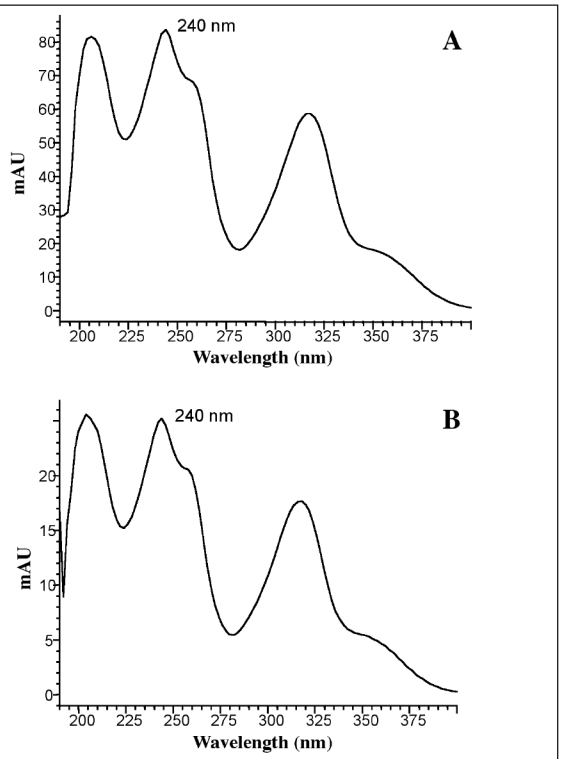

Figure 5. UV spectra of α-mangostin obtained on the PDA detector (corresponding to peaks in Figure 4): in standard solution (10 µg/mL) (A), in mangosteen peel extract sample solution (10 µg/mL) (B).

Linearity and calibration curve

Linearity of the method was confirmed by preparing standard curves for the analytical range of 1.0–20.0 µg/mL for determination of α-mangostin. The equation for the resultant calibration curve was $y = 84820x - 36104$; it showed a good correlation between analyte peak area and concentration of the α-mangostin on the analytical range with a linear regression coefficient of 0.9999.

The results of LOD and LOQ were found to be 0.02 and 0.08 µg/mL, respectively, which were lower than that of the previously published value in the literature (13) (0.13 and 1.33 µg/mL, respectively), indicating the better sensitivity of this proposed analytical method, which enables the determination of α-mangostin at low concentration in antibacterial film prepared from mangosteen peel extract.

Accuracy and precision

The intra-day precision (RSD) for three levels of standard α-mangostin concentrations (5.0, 10.0, and 15.0 µg/mL) was 1.8%, 0.7%, and 1.8%, respectively, with accuracy ranging from 98.1% to 100.8%, and that of inter-day analysis was 2.6%, 3.1%, and 4.3% with an accuracy ranging from 90.0% to 101.3%. All these data indicated good precision and accuracy. The accuracy and precision data are shown in Table I.

Table I. Intra- and Inter-Day Precision and Accuracy of the Method for Determination of α-Mangostin

	Intra-day* (n = 3)			Inter-day† (n = 9)		
CA‡ (µg/mL)	CF‡ (mean ± SD) (µg/mL)	RSD (%)	Accuracy (%)	CF‡ (mean ± SD) (µg/mL)	RSD (%)	Accuracy (%)
5.12	5.16 ± 0.09	1.8	100.8	5.19 ± 0.14	2.6	101.3
10.25	10.05 ± 0.07	0.7	98.1	10.15 ± 0.32	3.1	90.0
15.37	15.10 ± 0.27	1.8	98.2	15.33 ± 0.66	4.3	99.7

* Mean of triplicate analyses in a day.
† Mean of triplicate analyses per day over three days.
‡ CA = concentration added and CF = concentration found.

Recovery

The recovery of the method was tested by spiking the α-mangostin standards (at three different levels) into mangosteen peel extract sample, and then analyzing the mixture in triplicates per day over three different days. The resulting mean percentage recoveries were 95.8 ± 4.3%, 99.9 ± 4.1%, and 101.2 ± 2.3% at concentration levels of 5.0, 10.0, and 15.0 µg/mL, respectively; with the %RSD range 2.2–4.5, were considered acceptable (Table II).

Table II Recovery Studies of α-Mangostin in Mangosteen Peel Extract Sample

Spike level (µg/mL)	Recovery (%)*			Mean* (%)	RSD (%)
	Day 1	Day 2	Day 3		
5.08	100.8 ± 2.3	94.0 ± 1.4	92.8 ± 3.9	95.8 ± 4.3	4.5
10.16	104.6 ± 3.9	97.0 ± 3.8	98.2 ± 2.3	99.9 ± 4.1	4.1
15.24	102.9 ± 4.3	102.1 ± 4.7	98.7 ± 1.5	101.2 ± 2.3	2.2

* Mean ± standard deviation.

Conclusion

The method showed simplicity, good linearity, high precision and accuracy, and good recovery of the compounds of interest. This analytical method was proved to be a validated stability-indicating assay for α-mangostin in the presence of degradation products. The present method should be useful for analytical research and for routine quality control analysis

of α-mangostin in mangosteen peel extract and products of mangosteen such as antibacterial film prepared from mangosteen peel extract, which is now research in our laboratory.

Acknowledgments

The authors are grateful for the financial support to Prince of Songkla University Graduate School and Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat-Yai, Songkhla, Thailand.

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Manuscript received April 15, 2007;
revision received August 22, 2007.