HPLC Quantitative Analysis Method for the Determination of α-Mangostin in Mangosteen Fruit Rind Extract

W. Pothitirat and W. Gritsanapan*

Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University
Bangkok, 10400 Thailand

**Corresponding author. Email: pywgs@mahidol.ac.th*

Abstract

The fruit rind extract of mangosteen (*Garcinia mangostana* Linn.) of which a major active component is α-mangostin, has been popularly used in food supplements and herbal cosmetic preparations. α-Mangostin is used as a marker quantitative analysis and standardization of the raw materials and preparation from this plant. The precise method for analysis of plant constituents is normally a reverse-phase high performance liquid chromatographic (RP-HPLC). The aims of this study were to develop and validate a RP-HPLC method for determination of α-mangostin content in the extracts of mangosteen fruit rind. Chromatographic separation was carried out on a Hypersil® BDS C-18 column (4.6 x 250 mm, 5 μm) at room temperature using a gradient mobile phase consisting of 70-80 % acetonitrile in 0.1 %v/v ortho phosphoric acid at the flow rate of 1 mL min^{-1} with a UV detection at 320 nm. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ). The linearity of the proposed method was found in the range of 10–200 μg mL^{-1} with regression coefficient 0.9999. Intraday and interday precision studies showed the relative standard deviation less than 2 %. Accuracy of the method was determined by a recovery study conducted at 3 different levels, and the average recovery was 100.01 %. The LOD and LOQ were 0.06 and 0.17 μg mL^{-1}, respectively. Two samples of mangosteen fruit rind were separately extracted and analyzed using validated HPLC method. The contents of α-mangostin in the crude extracts and dried powder were within the ranges of 8.36 – 10.04 and 1.84 – 2.47 %w/w, respectively. This developed HPLC method was proven to be precise, specific, sensitive, and accurate for routine quality assessment of raw material of mangosteen fruit rind, its extract, and products.

Keywords: *Garcinia mangostana*, high performance liquid chromatography, mangostin, quality control, validation method

Introduction

At present, medicinal plants are employed throughout the industrialized and developing world as home remedies and ingredients for the pharmaceutical products. The lack of standardization and quality control for medicinal plants is still being problem. It seems to be necessary to determine the phytochemical constituents of herbal products in order to ensure the reliability and repeatability of pharmacological and clinical research to understand their bioactivities and possible side effects of active compounds and to enhance product quality control (Liang et al., 2004). For quality control of herbal products, high performance liquid chromatography (HPLC) is a popular method for the analysis of herbal medicines because it is accurate, precise and not limited by the volatility or stability of the sample compounds (Jandera et al., 2005; Holcapek et al., 2005; Klejdus et al., 2007; Hellström and Mattila, 2008; Lee et al., 2008).

Mangosteen or *Garcinia mangostana* Linn. belongs to the family Guttiferae and it is widely cultivated throughout Southeast Asian countries, especially in eastern and southern parts of Thailand. The fruit rind of this plant has long been used as a traditional medicine for treatment of abdominal pain, diarrhea, dysentery, infected wound, suppuration, and chronic ulcer (Mahabusarakam et al., 1987; Martin, 1980; Morton, 1987). It has been shown to contain a variety of phenolic compounds such as condensed tannins, anthocyanins, xanthones and their derivatives (Fu et al., 2007; Chin et al., 2008; Maisuthisakul et al., 2007). Several studies have reported that xanthones particularly α-mangostin (Figure 1), which is a major xanthone, exhibits antioxidant, antitumoral, anti-inflammatory, anti-allergic, antibacterial and antifungal activities (Jung et al., 2006; Chen et al., 2008; Chomnawang et al., 2007; Chomnawang et al., 2005; Gopalakrishnan et al., 1997; Chairungsrilerd et al., 1996; Iinuma et al., 1996; Ee et al., 2008; Nabandith et al., 2004). Due to its pharmacological activities, it is popularly applied to herbal cosmetics and pharmaceutical products. However, there is limited information for quality and quantity determination of α-mangostin in mangosteen extract. So, analytical methods play an important role in the quality control of its raw materials and products. From our previous study, we reported quantitative analytical methods of α-mangostin by UV-spectrophotometry and TLC-densitometry (Pothitirat and Gritsanapan, 2008a; Pothitirat and Gritsanapan, 2008b). These two methods are carried low cost in terms of solvents, number of samples and time consuming, but less in accuracy and precision. Therefore, the aims of this study were to develop and validate HPLC method for quantitative analysis of α-mangostin content in the mangosteen fruit rind extracts. HPLC fingerprints of the extract were also performed.

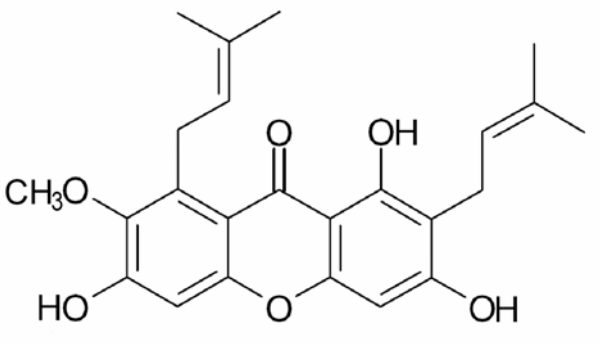

Figure 1 Chemical structure of α-mangostin.

Materials and Methods

Chemicals and Reagents

α-Mangostin was purchased from Chroma Dex Inc. (Santa Ana, CA). The other chemicals and solvents used in this experiment were analytical grade which were purchased from Labscan Asia (Thailand) and M&B Chemical Laboratory (England), except for 95 % ethanol which was obtained from the Excise Department, Bangkok, Thailand.

Plant Materials

The ripe fruits of *G. mangostana* were purchased from two local markets in Bangkok, Thailand in June 2006. The samples were identified by Dr. Wandee Gritsanapan, Faculty of Pharmacy, Mahidol University. The voucher specimens (WGM0614 and WGM0615) were deposited at the Department of Pharmacognosy, Faculty of Pharmacy, Mahidol University, Bangkok, Thailand. The fruits were cleaned and the edible aril part was removed. The fruit rinds were cut into small pieces and dried in a hot oven at 50 °C for 72 h. The dried samples were ground into powder, passed through a sieve (20 meshs). The samples were separately kept in air tight container and protected from light until used.

Instrumentation and Chromatographic Condition

HPLC method was performed on a Shimadzu SCL-10A HPLC system, equipped with a model LC-10AD pump, UV-vis detector SPD-10A, Rheodyne injector fitted with a 20 µL loop and auto injector SIL-10A. A Hypersil® BDS C-18 column (4.6 × 250 mm, 5 µm size) with a C-18 guard column was used. The elution was carried out with gradient solvent systems with a flow rate of 1 mL min^{-1} at ambient temperature (25-28°C). The mobile phase was consisted of 0.1 %v/v ortho phosphoric acid (solvent A) and acetonitrile (solvent B). The mobile phase was prepared daily,

filtered through a 0.45 μm and sonicated before use. Total running time was 37 min and the gradient programme was as follows: 70 % B for 0-15 min, 70 % B to 75 % B for 3 min, 75 % B to 80 % B for 1 min, constant at 80 % B for 6 min, 80 % B to 70 % B for 1 min. There was 11 min of post-run for reconditioning. The sample injection volume was 10 μL while the wavelength of the UV-vis detector was set at 320 nm. The compounds were quantified using CLASS VP software.

Preparation of Standard Solutions

A stock solution of α-mangostin reference standard (purity 97 %) was prepared by dissolving an accurately weighed 10 mg of α-mangostin in 10 mL of methanol in a volumetric flask. Various concentrations of the standard solution were diluted to obtain final concentrations at 200, 100, 50, 25, and 10 μg mL^{-1} with methanol.

Preparation of Sample Solutions

Each sample (10 g) was separately placed into a thimble and was extracted with 400 mL of 95% ethanol in a soxhlet apparatus for 15 h with 5 cycles h^{-1}. Each extract was filtered through a Whatman no. 1 filter paper by suction. The filtrate was concentrated under reduced pressured at 50°C using a rotary vacuum evaporator. The final weight of the crude extract was weighed and calculated for the yield. The extraction of each sample was done in triplicate.

Each dried extract (10 mg) was accurately weighed and transferred to a 10 mL volumetric flask. Methanol was added to volume (final concentration 1,000 μg mL^{-1}). Aliquot of the solution (2.5 mL) was diluted with methanol in a 10 mL volumetric flask to make a concentration of 250 μg mL^{-1}. Prior to analysis, the solutions were filtered through 0.45 μm membrane filters.

Quantitative Analysis of α-Mangostin Content

Determination of α-mangostin content was carried out by HPLC under the same condition as the proposed method. α-Mangostin content in the extract was calculated using its calibration curve with regarding to the dilution factor. The contents of α-mangostin in the extract and the fruit rind were expressed as gram per 100 grams of the extract and of the dried powder, respectively. Each determination was carried out in triplicate.

Validation of the Method

Validation of the analytical method was done according to the International Conference on Harmonization guideline (ICH, 1996). The method was validated for linearity, precision, accuracy, limit of detection (LOD) and limit of quantitation (LOQ).

Linearity

Linearity was determined by using α-mangostin standard solution of 1000 μg mL^{-1} in methanol. Ten to 200 μg mL^{-1} of the standard solution was prepared (n = 3). The calibration graphs were obtained by plotting the peak area versus the concentration of the standard solutions.

Precision

The precision was determined by analyzing 10, 25, and 50 μg mL^{-1} of standard solution of α-mangostin (n = 3) on the same day for intraday precision and on 3 different days for interday precision by the propose method. The precision was expressed as relative standard deviation (RSD).

Accuracy

The accuracy of the method was tested by performing recovery studies at 3 levels of α-mangostin reference standard added to the samples. Three different volumes (0.5, 1, and 1.5 mL) of the standard solution (containing 200 μg mL^{-1} of α-mangostin in methanol) were added to the sample solution (150 μg mL^{-1}) and analyzed by the proposed HPLC method. The recovery and average recovery were calculated. Three determinations were performed for each concentration level.

Limit of Detection (LOD) and Limit of Quantitation (LOQ)

According to the International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use recommendations, the approach based on SD of the response and the slope were used for determining the detection and quantitation limits.

Results and Discussion

HPLC method with gradient elution was developed for the quantification of α-mangostin in *G. mangostana* fruit rind extracts. The mixture of 0.1 % ortho phosphoric acid and acetonitrile gave optimum chromatographic separation of α-mangostin with the other peaks in the extract (Figure 2). It was found that the resolution was very good (resolution value > 1.5). The wavelength at 320 nm was used for all measurements due to its maximum absorption.

The method was validated for its linearity, precision, accuracy, LOD, and LOQ. The calibration graph for α-mangostin was within the concentration range of 10 – 200 μg mL^{-1}, with a correlation coefficient (r^2) of 0.9999 (Table 1). The interday and intraday precisions of α-mangostin are presented in Table 2. The results showed acceptable precision of the method, with RSD values lower than 2%. The recovery at 3 different levels of α-mangostin was 105.10, 95.00, and 99.93 %, with an average of 100.01% (Table 3).These values indicate the accuracy of the method. The LOD and LOQ for α-mangostin were found to be 0.06 and 0.17 μg mL^{-1}, respectively, which indicate a high sensitivity of the method (Table 1).

Table 1 Method validation parameters for the quantification of α-mangostin by the proposed HPLC method.

Parameters	Results
Linear range (μg mL^{-1})	10-200
Regression equation[1/]	y = 33674x - 7244.7
Correlation coefficient (r^2)	0.9999
LOQ (μg mL^{-1})	0.17
LOD (μg mL^{-1})	0.06

[1/] x is the concentration of α-mangostin in μg mL^{-1}, y is the peak area at 320 nm

Table 2 Intraday and interday precision of α-mangostin determination by the proposed HPLC method.

Concentration	Intraday precision	Interday precision
(μg mL^{-1})	(---------- % RSD---------)	
10	1.97	0.77
25	0.61	1.65
50	1.21	1.63

Table 3 Recovery study of α-mangostin by the proposed HPLC method.

Serial no.	Amount present in the extract	Amount added	Amount found[1/]	Recovery[1/]
	(---------- μg mL^{-1} ----------)			(%)
1	0.26	10	10.77 ± 0.07	105.10 ± 1.21
2	0.26	20	19.26 ± 0.34	95.00 ± 1.81
3	0.26	30	30.26 ± 1.58	99.93 ± 1.51
		Average		100.01

[1/] Expressed as mean ± standard deviation (SD; n = 3).

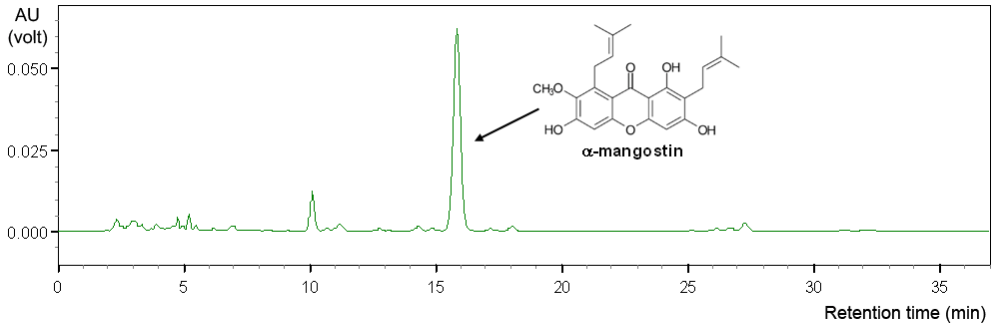

Figure 2 HPLC fingerprint of *G. mangostana* fruit rind extract.

Table 4 Yield of crude extract and the content of α-mangostin in the extracts and dried powder of *G. mangostana* fruit rinds by the proposed HPLC method.

Sample	Yield of crude extract[1/]	α-Mangostin content[1/]	
		In dried powder	In extract
	(% w/w of dried powder)	(---------- % w/w----------)	
Local market A	24.41 ± 0.21	2.47 ± 0.08	10.04 ± 0.33
Local market B	22.46 ± 0.95	1.82 ± 0.04	8.36 ± 0.17

[1/] Expressed as mean ± standard deviation (SD; n = 3).

α-Mangostin content in the samples of *G. mangostana* fruit rind which obtained from different local markets in Bangkok during June 2006 determined by the developed HPLC method is given in Table 4. The contents of α-mangostin in the ethanolic extracts of Sample A and Sample B were 10.04 ± 0.33 and 8.36 ± 0.17 % w/w, while in the dried powder were 2.47 ± 0.08 and 1.82 ± 0.04%w/w, respectively (Table 4). HPLC chromatograms of both extracts showed similar pattern with a major peak of α-mangostin at retention time of 16.32 min (Figure 2). The identity of the peak of α-mangostin in the sample chromatograms was confirmed by spiking with its standard and determination of retention time.

Yodhnu et al. (2009) reported that the isocratic RP-HPLC method can be used for quantitative determination of α-mangostin in the extract from *G. mangostana*. In our work, we used gradient RP-HPLC which offers advantages over isocratic RP-HPLC for separation a wide range of compounds with high resolution. Comparing with other analytical methods such as TLC-densitometric and UV- spectrophotometric methods, HPLC promotes higher precision, accuracy and sensitivity. Although a UV spectrophotometry is a rapid and economical method but it does not give information of each component of mangostins, i.e., α-mangostin, while HPLC can be used for separation, identification and quantification of individual mangostin. TLC-densitometry is appropriate when a lot of samples are needed to be analyzed routinely, and not high accuracy is required.

Conclusions

The proposed HPLC method promoted high precision, sensitivity and accuracy for quality control of raw materials of *G. mangostana* fruit rind and its extract. It should be useful for quantitative analysis of α-mangostin content in the products derived from *G. mangostana* fruit rind extracts such as anti-acne mangosteen gel which is now carried out in our laboratory. α-Mangostin is recommended for using as a marker for mangosteen analysis due to its majority and biological activities. This proposed method will be useful for quantitative analysis in standardization and quality assessment of *G. mangostana* fruit rind extracts for pharmaceutical and cosmetic uses.

Acknowledgments

This study is a part of a Ph.D. thesis of Mahidol University, Bangkok, Thailand and was granted by the University Research Fund.

References

Chairungsrilerd, N., K. Furukawa, T. Ohta, S. Nozoe and Y. Ohizumi. 1996. Histaminergic and serotonergic receptor blocking substances from the medicinal plant *Garcinia mangostana*. Planta Med. 62: 471-472.

Chen, L.G., L.L. Yang and C.C. Wang. 2008. Anti-inflammatory activity of mangostins from *Garcinia mangostana*. Food Chem. Toxicol. 46: 688-693.

Chin, Y.W., H.A. Jung, H. Chai, W.J. Keller and A.D. Kinghorn. 2008. Xanthones with quinone reductase-inducing activity from the fruits of *Garcinia mangostana* (Mangosteen). Phytochemistry 69: 754-758.

Chomnawang, M. T., S. Surassmo, V.S. Nukoolkarn and W. Gritsanapan. 2005. Antimicrobial effects of Thai medicinal plants against acne-inducing bacteria. J. Ethnopharmacol. 101: 330-3.

Chomnawang, M. T., S. Surassmo, V.S. Nukoolkarn and W. Gritsanapan. 2007. Effect of *Garcinia mangostana* on inflammation caused by *Propionibacterium acnes*. Fitoterapia 78: 401-408.

Ee, G.C., S. Daud, S.A. Izzaddin and M. Rahmani. 2008. *Garcinia mangostana*: a source of potential anti-cancer lead compounds against CEM-SS cell line. J. Asian Nat. Prod. Res. 10: 481-485.

Fu, C., A.E.K. Loo, P.P. Chia and D. Huang. 2007. Oligomeric proanthocyanidins from mangosteen pericarps. J. Agric. Food Chem. 55: 7689-7694.

Gopalakrishnan, G., B. Banumathi and G. Suresh. 1997. Evaluation of the antifungal activity of natural xanthones from *Garcinia mangostana* and their synthetic derivatives. J. Nat. Prod. 60: 519-524.

Hellström, J.K. and P.H. Mattila. 2008. HPLC determination of extractable and unextractable proanthocyanidins in plant materials. J. Agric. Food Chem. 56: 7617-7624.

Holcapek, M., M. Lísa, P. Jandera and N. Kabátová. 2005. Quantitation of triacylglycerols in plant oils using HPLC with APCI-MS, evaporative light-scattering, and UV detection. J. Sep. Sci. 28: 1315-1333.

ICH Steering Committee. 1996. International Conference on Harmonization of Technical Requirements for the Registration of Pharmaceuticals for Human Use. Validation of analytical procedure: Methodology.

Iinuma, M., H. Tosa, T. Tanaka, F. Asai, Y. Kobayashi, R. Shimano and K. Miyauchi. 1996. Antibacterial activity of xanthones from guttiferaeous plants against methicillin-resistant *Staphylococcus aureus*. J. Pharm. Pharmacol. 48: 861-865.

Jandera, P., V. Skeifiková, L. Rehová, T. Hájek, L. Baldriánová, G. Skopová , V. Kellner, V and Horna, A. 2005. RP-HPLC analysis of phenolic compounds and flavonoids in beverages and plant extracts using a CoulArray detector. J. Sep. Sci. 28: 1005-1022.

Jung, H.A., B.N. Su, W.J. Keller, R.G. Mehta and D. Kinghorn. 2006. Antioxidant xanthones from the pericarp of *Garcinia mangostana* (mangosteen). J. Agric. Food Chem. 54: 2077-2082.

Klejdus, B., J. Vacek, L. Benesová, J. Kopecký, O. Lapcík and V. Kubán. 2007. Rapid-resolution HPLC with spectrometric detection for the determination and identification of isoflavones in soy preparations and plant extracts. Anal. Bioanal. Chem. 389: 2277-2285

Lee, S.T., D.R. Gardner, C.W. Chang, K.E. Panter and R.J. Molyneux . 2008. Separation and measurement of plant alkaloid enantiomers by RP-HPLC analysis of their Fmoc-Alanine analogs. Phytochem. Anal. 19: 395-402.

Liang, Y.Z., P. Xie and K. Chan. 2004. Quality control of herbal medicines. J. Chromatogr. B. 812: 53-70.

Mahabusarakam, W., P. Iriyachitra and W.C. Taylor. 1987. Chemical constituents of *Garcinia mangostana*. J. Nat. Prod. 50: 474-478.

Maisuthisakul, P., M. Suttajit and R. Pongsawatmanit. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chem. 100: 1409-1418.

Martin, F.W. 1980. Durian and mangosteen, pp. 407–414. *In* S. Nagy and D.S. Shaw, eds., Tropical and Subtropical Fruits: Composition, Properties and Uses.

Morton, J. 1987. Mangosteen: *Garcinia mangostana* L., pp. 301-304. *In* F. Julia, ed., Fruits in Warm Climates.

Nabandith, V., M. Suzui, T. Morioka, T. Kaneshiro, T. Kinjo, K. Matsumoto, Y. Akao, M. Iinuma and N. Yoshimi. 2004. Inhibitory effects of crude alpha-mangostin, a xanthone derivative, on two different categories of colon preneoplastic lesions induced by 1, 2 dimethylhydrazine in the rat. Asian Pac. J. Cancer Prev. 5: 433-438.

Pothitirat, W. and W. Gritsanapan. 2008a. Thin layer chromatography densitometric analysis of α-mangostin content in *Garcinia mangostana* fruit rind extracts. J. AOAC Int. 91: 1145-1148.

Pothitirat, W. and W. Gritsanapan. 2008b. Quantitative analysis of total mangostins in *Garcinia mangostana* fruit rind. J. Health Res. 22: 161-166.

Yodhnu, S., A. Sirikatitham, and C. Wattanapiromsakul. 2009. Validation of LC for the determination of α-mangostin in mangosteen peel extract: A tool for quality assessment of *Garcinia mangostana* L. J. Chromatogr. Sci. 47: 185-189.

Manuscript received 5 March 2009, accepted 3 June 2009