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RP-HPLC Method Validation for Purity Assay of α-Mangostin Isolate

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ABSTRACT: *The quality of natural products regarding the purity of their active compounds, such as α-mangostin isolate from mangosteen pericarp, may vary depending on cultivation, harvest season, and isolation process. Thus, extensive isolation procedures are involved in obtaining a certain level of purity of the active compounds. Studies on the yield of α-mangostin isolate and its effectiveness as an active compound in health care have been reported. The quality parameter of the isolate as the intended active compound can be indicated by its purity level. Measuring the purity of the active compound is proposed to define the grade α-mangostin isolate as a starting material or even reference standard. The higher the purity level of α-mangostin isolate, the greater its potential as a reference standard candidate. Therefore, a selective analytical method is required to measure the purity level accurately. For this reason, a rapid analytical method to ensure α-mangostin isolate was developed and validated to confirm its purity. Separation condition semployed an X-Terra® C18 column 5 µm, 4.6 x 150 mm under an isocratic system with a mobile phase composition of MeCN:water (85:15) at a flowrate of 0.5 mL/min and a detector wavelength of 243 nm were selected. Acceptable validation parameters of linearity in the range of 2.6 –52 µg/mL with r^2 = 0,9994, Vx_0 = 2.64%; accuracy 96.38 – 100.99%; precision 1.36%; and LOD/LOQ = 4.6 µg/mL/ 13.7µg/mL were achieved. The validated method was successfully applied to the purity assay α-mangostinisolate with a run time of less than 9 minutes.*

Keywords: *α-mangostin; isolate; method validation; purity assay; RP-HPLC*

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1. Introduction

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit locally cultivated in several Southeast Asian countries, including the Philippines, Thailand, Malaysia, and Indonesia. The fruit pericarp (rind) of mangosteen is widely used as a natural compound in medication [1]. Known as the queen of fruits, the appearance of purple-dark brown pericarp hints at its antioxidant content. Antioxidant properties of mangosteen are polyphenolic compounds containing xanthones groups, namely α-mangostin, β-mangostin, and ɣ-mangostin as shown in Figure 1 [2,3].

The benefits of α-mangostin in health care have been scientifically proven. Pedraza-Chaverri*et al* (2009) reported the antioxidant potency of α-mangostin towards mitochondrial toxin 3-nitropropionic acid (3-NP) [4]. Furthermore, its potential renoprotection [5], curing acute renal injury by inhibiting reactive oxygen species (ROS) production of the dead cells [6], and as a therapeutic agent against liver fibrosis [7] has been studied. However, as they are derived from natural resources, harvest seasons and climate may affect α-mangostin content and possibly lead to varying quantity levels. This means several bulk and final nature-based commercial products in the market must be monitored to ensure their-quality and safety.

Studies on the determination of α-mangostin in-mangosteen pericarp extract using HPLC-UV/Vis, HPLC-PDA, and LC-MS have been reported [8,9]. A study on mangosteen peel extract as a potential antioxidant and antiaggregation by HPLC has been reported [10]. In addition to the determination of extracts, the further isolation steps of the intended active compounds and their evaluation maybe more challenging due to the laborious process and, on the other hand, the limited yield of the isolate. Liao *et al* (2023) reported the yield of α-mangostin isolate obtained in their study of about 5% [7]. Thus, sample handling and the selection of analytical approaches to evaluate the active compound isolate are also critical. A simple, rapid, and selective analytical method is required in the purity test of α-mangostin isolate, which also relates to its active compound assay.

Chromatography is an established technique that possesses rapid separation in relatively low sample consumption. It is widely applied for many purposes of analysis, including quality control of natural products, such as impurity tests and determining the active compounds. A study by Pilkington et al (2012) reported that impurities have a critical impact on the quantification of artemisinin, which may also be the case for any natural extracts and isolates [11]. Furthermore, the purity assay of α-mangostin isolate using HPLC, therapeutic effect, and cytotoxicity was evaluated [12]. An advanced application of analytical technique using UPLC-ESI/MS for quantification of α-mangostin in dietary supplements has been reported [13]. Among the employed chromatography techniques for the active compound assay, including α-mangostin [8,11,12,14], only a few studies reported the application in the isolates [12]. Especially, the previous reported studies were not specific purpose of finding standard reference candidates by measuring the purity of the intended isolates. This study offers an alternative RP-HPLC method for the assay of α-mangostin isolate to the existing reported methods as a part of finding standard reference candidate.

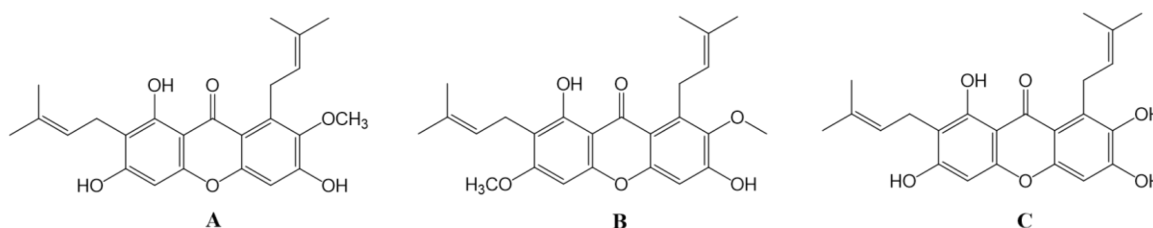

Figure 1. Structure of α-mangostin (A), β-mangostin (B), ɣ-mangostin (C)

2. Materials and methods

2.1. Chemicals and reagents

The α-mangostin reference standard ≥ 98% (HPLC) was purchased from Supelco® (Saint Louis, USA). The tested sample of α-mangostin isolate was kindly provided by Sekolah Tinggi Farmasi Indonesia (STFI, Bandung, Indonesia). Methanol (MeOH) and acetonitrile (MeCN) (gradient grade for liquid chromatography) were acquired from Merck KGaA (Darmstadt, Germany). Water was purified by Direct-Q® 0.2 μm water purification systems from Merck KGaA (Darmstadt, Germany).

2.1. HPLC instrumentation

The method was developed using HPLC Waters® Alliance 2695e and UV detector 2489. Data acquisition was performed by Empower™ 2 Software, involving integration parameters of peak area, symmetry factor, and retention time. Separation condition was performed using an X-Terra® C18 column 4.6 x 150 mm Waters at a column temperature of 25°C, a mobile phase of MeCN:water (85:15), and a flow rate of 0.5 mL/min. The injection volume of 20 μL was set at a detection wavelength of 243 nm.

2.3. Sample preparation

The assay of α-mangostin isolate was conducted using a calibration curve prepared from a stock solution in MeOH. The calibration curve of α-mangostin consisted of six concentrations in the range of 2.6 – 52μg/mL. The precision and accuracy of the method were performed using three different concentration of triplicate injection (*n* = 9). An in-house isolate of α-mangostin was selected as the sample, and its purity was determined.

3. Data evaluation

The validation parameters were calculated based on ICH guidelines [15]. The limit of detection (LOD) and limit of detection (LOD) were evaluated as follows Equation (1) and Equation (2) [16,17]:

Equation (1) $$LOQ = 3.3\frac{\sigma}{S}$$

Equation (2) $$LOQ = 10\frac{\sigma}{S}$$

σ = the standard deviation of the response; S = the slope of the calibration curve, also known as *b*. The slope was estimated from the regression line of six working solution concentrations (2.6 – 52 μg/mL). A linear correlation was represented by a coefficient correlation in Equation (3) and Equation (4)[16,17].

Equation (3) $$y = bx + a$$

Equation (4) $$V_{x0} = \frac{Sx_0}{\bar{x}}$$

Sx_0 and Sy were calculated using Equation (5) and Equation (6), respectively [16].

Equation (5) $$S_{x0} = \frac{Sy}{b}$$

Equation (6) $$Sy = \sqrt{\frac{\sum(y-\hat{y})^2}{n-2}}$$

$\bar{x}$ is the x-axes average (the mean of standard concentrations), Vx_0 is the coefficient variation Sx_0, and is the standard deviation of the function. While *n* is the number of working solutions.

In this study, the peak integration and symmetry factor were performed by Empower™ 2 Software. The formula applied to evaluate the USP symmetry factor (A_s) is depicted in Equation (7) [17].

Equation (7) $$A_s = \frac{W_{0.05}}{2d}$$

$W_{0.05}$ = the width of the peak at one-twentieth of the peak height; d = the distance between perpendicular dropped from the peak maximum to the edge of the peak at one-twentieth of the peak height [17].

4. Results and discussion

The system suitability tests showed consistent performance of the HPLC system based on the repetitive retention times, an average of 7.708 minutes with an acceptable relative standard deviation (RSD) of 0.35%. In addition, the symmetry factor showed an acceptable range of peak tailing (USP tailing), which was not more than 2. The chromatographic parameters were listed in Table 1.

The symmetry factor represented Gaussian peak shape with an ideal value of 1. The resulting chromatograms' symmetry factor showed an average of 1.343, meaning a tailing peak appears. However, the symmetry value was acceptable within the limit of ≤ 2. Furthermore, system suitability performance showed an excellent t_R precision with a relative standard deviation (RSD) of 0.35%.

The validation method was performed in the 2.6 – 52 μg/mL concentration range to evaluate the linearity, as shown by r^2 and Vx_0 parameters. The method linearity was indicated by a correlation coefficient of r^2 0.9994 ≥ 0.9990 and regression function coefficient Vx_0 2.64% ≤ 5%. The method accuracy and precision at standard addition concentrations of 10.4 μg/mL; 13.0 μg/mL; and 15.6 μg/mL in triplicate injections showed an acceptable value of 96.38-100.99% and 1.36% RSD, respectively. Validation parameters of LOD, LOQ, linearity, range, accuracy, and precision are depicted in Table 2.

The validated method was applied for the purity assay of α-mangostin isolate from in-house isolation of mangosteen pericarp. The peak of the mangostin isolate was confirmed by the α-mangostin standard with the t_R at about 7.7 minutes, as shown in Figure 2.

The purity assay was conducted in three replicate concentrations, as depicted in Table 3 and shown by the chromatogram profile in Figure 3.

The tested α-mangostin isolates showed a baseline peak without noise interference. The peak at the lowest concentration shifted to the later retention time. This phenomenon might appear due to minor conditions affecting the interaction between the analyte and the stationary phase, such as pressure and gas in the chromatography system. However, the retention time pre-

Table 1. Sistem suitability and peak quality

Parameter	Value ($\bar{x}$ ± *SD*)
t_R(min)	7.708 ± 0.027
Symmetry factor (%)	1.343 ± 0.006

n = 9 (three different concentrations)

Table2. Validation method parameters

Parameter	Value
Specificity	No interference
LOD/LOQ	4.6 / 13.7μg/mL
Linearity	
r^2	0.9994
Vx_0	2.64%
Range	2.6 – 52μg/mL
Accuracy	96.38 – 100.99%
Precision	1.36%

accuracy and precision of three different concentrations (*n* = 9)

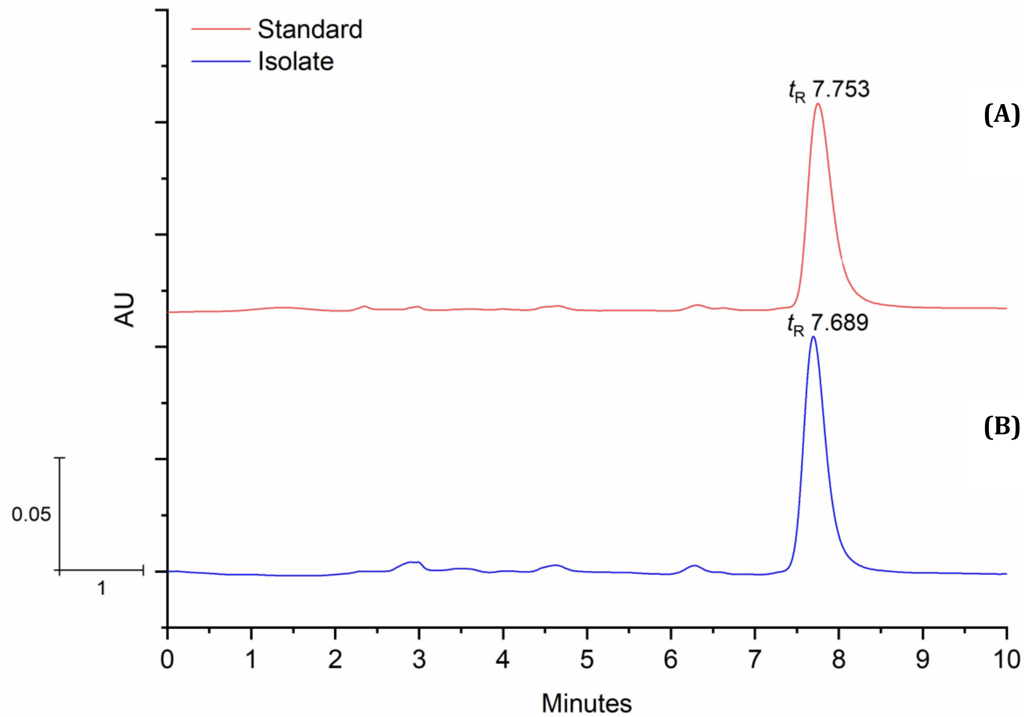

Figure 2. Chromatograms of α–mangostin standard at 18.2 µg/mL (A) and the tested isolate at 15.0 µg/mL (B) at analysis conditions: X-Terra® C18 column 4.6 x 150 mm 25°C, mobile phase of MeCN:water (85:15), flow rate of 0.5 mL/min, injection volume 20 µL, and detection wavelength of 243 nm.

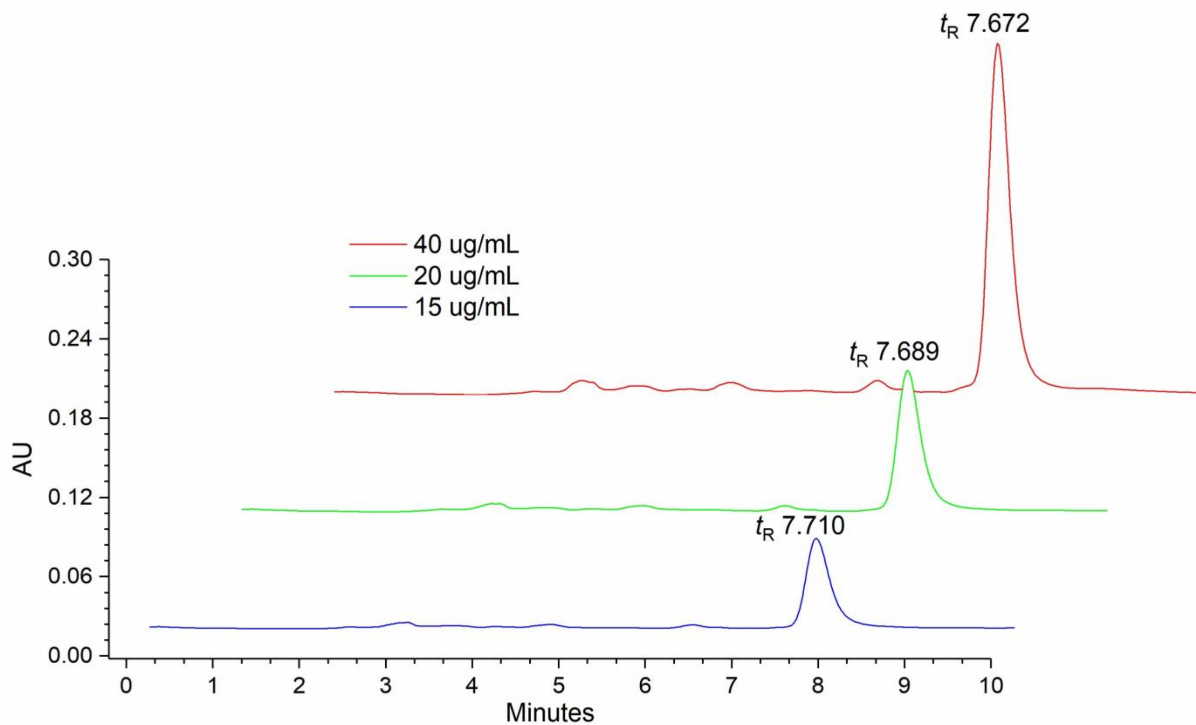

Figure 3. Chromatograms of α–mangostin isolate in three concentrations at analysis conditions: X-Terra® C18 column 4.6 x 150 mm 25°C, mobile phase of MeCN:water (85:15), flow rate of 0.5 mL/min, injection volume 20 µL, and detection wavelength of 243 nm

cision of 0.25% RSD was obtained. This means, that the shifted peak was still acceptable for the retention time precision. Furthermore, the selected sample concentrations showed a repetitive proportional response.

5. Conclusions

The proposed method fulfills the required validation parameters of linearity with r^2 0.9994; $Vx_0$of 2.64% within concentrations 2.6 – 52 µg/mL range, accuracy of 96.38 – 100.99%, precision of 1.36%, LOD/LOQ of 4.6µg/mL/13.7 µg/mL and is applicable as an alternative method for the purity test of α-mangostin isolate with rapid separation less than 9 minutes, in a low sample consumption.

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