HPLC Method Optimization of α-Mangostin Assay in Mangosteen 
(Garcinia mangostana L.) Fruit Rind Extract 
Formulated in Oral Solution

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Abstract

Antioxidant from natural ingredients is important to reduce an incident of degenerative diseases and to prevent free radicals. One of them is mangosteen fruit rind which is rich in antioxidants, especially α-mangostin as a major component. The developed formulation of oral solution contains mangosteen fruit rind extract that needs an assay method for quality testing. The α-mangostin compound can be used as chemical marker. To determine a very low concentration of analyte in sample with very complex matrix, such as in oral solution, it needs a selective and sensitive method, that can be obtained in high performance liquid chromatography (HPLC). In this study, α-mangostin assay was performed by RP-HPLC system using octadecylsilane (C18) stationary phase with various ratios of mobile phase and flow rate. The best result are given by system with methanol-water (90:10) as mobile phase, flow rate of 1.0 mL/min, and UV detector at 316 nm. In addition, the retention time of α-mangostin was 9.622 minutes.

Keywords: α-mangostin, Garcinia mangostana L., assay, oral solution, HPLC

INTRODUCTION

One of the compound from Garcinia mangostana rind extract that has antioxidant activity is α-mangostin. Oral solution formulation of Garcinia mangostana rind extract has been done using cosolvency method. Based on α-mangostin solubility the best chosie was obtained with PEG 400 and glycerin of 40% (Sofiah et al., 2013). In this oral solution there are Garcinia mangostana rind extract and excipient that are very complex. To analyse the α-mangostin in this oral solution, it need such a specific and sensitive method. It is high performance liquid chromatography (HPLC). This method had been used to assay α-mangostin in Garcinia mangostana rind extract (Aisha et al., 2013; Yodhnu et al., 2009; Pothitirat and Gritsanapan, 2009; Islam and Begum, 2011; Jujun et al., 2009), after extract encapsulation in PLGA (Poly Lactic-co-Glycolic Acid) microspheres (Ali et al., 2012), and in plasma blood rat (Syamsudin et al., 2010). In this research, optimation of HPLC condition was done using various mobile phase, flow rate and detector.
MATERIAL AND METHODS

α-Mangostin standard compound was purchased from Chengdu Biopurify Phytochemicals Ltd. Oral solution of mangosteen (*Garcinia mangostana* L.) fruit rind extract was prepared using cosolvency methods (Sofiah *et al.*, 2013).

**Determination of maximum absorption of α-mangostin**

Determination of maximum absorption was conducted with recording of absorption spectra at 20 ppm of α-mangostin standard solution in the region of 200-500 nm using Shimadzu UV-1800 spectrophotometry.

**Determination of HPLC optimum condition**

This assay was conducted using Shimadzu LC-20AD, with octadecylsilane (C18) Shim-pack VP-ODS 9.4 x 250 mm; 4.6 μm at the temperature of 40°C. Based on previous research (Aisha *et al.*, 2013; Yodhnu *et al.*, 2009; Pothitirat and Gritsanapan, 2009; Islam and Begum, 2011; Jujun *et al.*, 2009; Teixeira *et al.*, 2003) the various mobile phase and flow rate were performed to choose the best condition. The solvent system are acetonitrile-0.1% of ortho phosphoric acid in water and methanol-water.

RESULTS AND DISCUSSION

**Maximum wavelength of α-mangostin**

UV spectrum of α-mangostin in the region of 200-500 nm are seen on Fig. 1.

![Figure 1. UV spectra of α-mangostin standard solution](image-url)
There are 2 maximum wavelength, namely 243.2 nm and 316.4 nm. These two maximum wavelength were similar with those of Aisha et al. (2013). The wavelength of 316 nm were choosed as detector in HPLC because the use of 244 nm as detector could disturb baseline stabilization, raise such noise and ghost peak when using methanol as mobile phase. In addition, 316.4 nm need lower energy than 243.2 nm.

**Determination of HPLC optimum condition**

Optimum condition was made from various mobile phase using the flow rate of 1.0 mL/minute. If there is low resolution, the flow rate was 0.8 mL/minute and if the retention time was long it was adjusted in 1.2 mL/minute. The column was the same in all condition namely octadecylsilane (C18) Shim-pack VP-ODS with dimension of 4.6 x 250 mm; 4.6 μm, column temperature of 40°C, and using 316 nm as detector.

First experiment was done using mobile phase of acetonitrile-0.1% ortho phosphoric acid in water (95:5). The result could be seen as follows.

Fig 2. Showed that with flow rate of 1.0 ml/minute the retention time of α-mangostin was 5.331 minute. It was quite fast. In oral solution chromatogram, α-mangostin peak wasn’t separated with the other compounds. α-Mangostin standard chromatogram showed two peaks. Using 0.8 ml/minute of flow rate, α-mangostin has two peaks with the retention time of major peak was 6.664 minute (Fig. 3 a). The peak of α-mangostin in the oral solution is not separated well. So It was not the candidate of the mobile phase for HPLC.
a. Figure 3. α- mangostin (a) and oral solution chromatograms (b) using mobile phase of acetonitrile- 0.1% ortho phosphoric acid in water (95:5) with flow rate of 0.8 mL/minute

The next experiment was conducted using mobile phase of acetonitrile-0.1% ortho phosphoric acid in water (75:25). The chromatograms were showed as Fig 4 and Fig. 5.

a. Figure 4. α- mangostin (a) and oral solution chromatograms (b) using mobile phase of acetonitrile-0.1% ortho phosphoric acid in water (75:25) with flow rate of 1.0 mL/minute

b. Figure 5. α- mangostin (a) and oral solution chromatograms (b) using mobile phase of acetonitrile-0.1% ortho phosphoric acid in water (75:25) with flow rate of 1.2 mL/minute
Fig 4. Shows that α-mangostin chromatogram has the retention time of 14.123 minute. α-mangostin peak was separated well with the others (b) but α-mangostin peak was splitted. To know whether the retention time of α-mangostin will be shorter and still well separated the flow rate is conducted in flow rate of 1.2 mL/minute. In this condition α-mangostin has the retention time of 11.848 minute (Fig. 5 a), well separated in the oral solution but there is another peak in α-mangostin standard chromatogram. Tailing could interfere the α-mangostin area so that in the assay there is no accuracy.

The other mobile phase was methanol-water (95:5). In this condition α-mangostin peak was detected with a retention time of 5.797 minute for reference standards (Fig. 6 a) and 5.990 minute for oral solution (Fig. 6 b). The retention time is quite fast. However, the α-mangostin peak in the oral solution are not separated properly. In addition, the tailing factor of α-mangostin peaks on the chromatogram reference standard is too large, ie 2.085 (more than 2.0). Tailing factor that can generate large peak area values are less accurate. Slower flow rate was applied to see if α-mangostin peak in the oral solution can be well separated and the tailing factor of α-mangostin peaks on the reference standard chromatogram may be lower.

Experiments using this mobile phase with a flow rate of 0.8 mL/minute generated chromatogram as seen in Fig 7. α-Mangostin peak was detected with a retention time of 7.343 minute for reference standard (Fig. 7 a) and 7.441 minute for oral solution (Fig 7 b). Retention time is still quite fast. However, the peak of α-mangostin in the oral solution is still not well separated although the tailing factor of α-
mangostin peaks on the chromatogram reference standard has been reduced to 1.741 (less than 2.0). Thus, the mobile phase can not be a candidate for the selected mobile phase.

Figure 7. α-mangostin (a) and oral solution chromatograms (b) using mobile phase of methanol-water (95:5) with flow rate of 0.8 mL/minute

Another tested mobile phase was methanol-water (90:10). Experiments using this mobile phase with a flow rate of 1.0 mL / minute generated chromatogram as follows.

α-Mangostin peak was detected with a retention time of 9.622 minute for reference standards and 9.807 minute for oral solution (Fig 8 a and b). Peak of α-mangostin in the oral solution was well separated with resolution of 1.725 (better than 1.5) that fulfill the criteria of the specificity test. The tailing factor of α-mangostin peaks on the reference standard chromatogram is 1.213 and 1.378 on the oral solution chromatogram. The faster flow rates was tested in order the retention time of α-
mangostin can be more quick and remain well separated in the oral solution. Using the same mobile phase with flow rate of 1.2 mL/minute, generated chromatogram was follows.

α-Mangostin peak was detected with a retention time of 8.230 minute for reference standard (Fig. 9 a) and 8.342 minute for oral solution (Fig. 9.b). Peak of α-mangostin in the oral solution remains well separated with resolution more than 1.5, the tailing factor of α-mangostin peaks on the reference standard chromatogram was 1.269 and 1.291 on the oral solution. Thus, the mobile phase may be a candidate for the selected HPLC mobile phase.

Based on 4 mobile phases, methanol-water (90:10) was the only candidate HPLC mobile phase for selected conditions. This is advantageous because composition of methanol in the mobile phase is lower than the mobile phase of methanol-water other tried and not using acetonitrile (less expensive and less toxic). In addition, the mobile phase is simple because it contains only two components (methanol and water) and do not need the buffer.

Flow rate tested with the mobile phase was 1.0 mL/minute and 1.2 mL/minute. The results showed a good separation (high resolution) and low tailing factor. HPLC conditions used for the selected flow rate 1.0 mL/minute with the following considerations. Burden of pumps used is more heavily if the flow rate is 1.2 mL/minute. On the use of a flow rate of 1.0 mL/minute time required for data acquisition (acquisition time) was 12.5 minute while the use of a flow rate of 1.2 mL/minute is 11 minute. The volume of the mobile phase with a flow rate of 1.0 mL/minute was 12.5 mL and a flow rate of 1.2 mL/minute needs 13.2 mL of mobile
phase. Therefore, for routine analysis would be more efficient to use a flow rate of 1.0 mL / minute.

CONCLUSION
The optimum HPLC conditions that can be used for analysis of α-mangostin in the oral solution of mangosteen fruit rind extract are octadecylsilane stationary phase (C18), mobile phase of methanol-water (90:10) with flow rate of 1.0 mL / minute, and detector of 316 nm.

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