The validation HPLC method for determination of gambogic acid in gamboge resin

E M Khaing1, K Jitrangsri2, J Mahadlek3, C Sukonpan4 and T Phaechamud1,5*

1Program of Pharmaceutical Engineering, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
2Program of Pharmaceutical Sciences, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
3Pharmaceutical Intellectual Unit “ Prachote Plengwittaya”, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
4Department of Pharmaceutical Chemistry, Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand
5Natural Bioactive and Material for Health Promotion and Drug Delivery System Group (NBM), Faculty of Pharmacy, Silpakorn University, Nakhon Pathom, Thailand

*Corresponding author: phaechamud_t@su.ac.th

Abstract. Gambogic acid obtained from plant resin of Garcinia species has anticancer properties. This study focuses on the development and validation of the high-performance liquid chromatography (HPLC) method for the quantification of gambogic acid (GA) content in the gamboge raw sample. The chromatographic system was performed on C18 column (150 × 4.6 mm, 5 µm). The system consisted of acetonitrile and 0.1% orthophosphoric acid (85:15 v/v) as mobile phase with a flow rate of 1.5 mL/min. The detection was carried out at a wavelength of 360 nm and the retention time of GA was evident at 9 min. The method was validated according to ICH guidelines. The obtained calibration plot exhibited good linearity in the range of 5 to 120 µg/mL (r² > 0.999). The percentage recovery GA spiked in the gamboge sample ranged from 98.87% to 102.92%. The LOD and LOQ were shown to be 2.069 and 6.271 µg/mL, respectively.

The validation study demonstrated that this method was simple, specific, and applicable for quantitative analysis of GA in gamboge resin.

1. Introduction
Gamboge, plant resin is obtained from the Garcinia species such as Garcinia hanburyi Hook.f., has been utilized in colour pigmentation and folk medicine for the treatment of pain, edema, infected wound and laxative [1]. Gambogic acid (GA) is the caged xanthone family and is isolated from gamboge resin as the main active compound. Its antitumor activities are interested in pharmaceutical applications. GA has a potent anticancer effect against various cancer cells containing liver, breast, lung, colon, prostate and ovarian cancer [2]. The mechanism of GA on colon cancer SW620 cells was due to the suppressing of cell proliferation and dispersion in a dose-dependent effect in vitro assay [3].

HPLC method was explored for the determination of eight polyprenylated xanthone compounds in gamboge resin including gambogic acid [4]. The determination of GA and gambogenic acid in rat...
plasma could be conducted using liquid chromatography–tandem mass spectrometry [5]. A non-aqueous capillary electrophoresis method has been developed to determine gambogenic acid in gamboge [6]. However, only GA content analysis in gamboge resin using HPLC method has not been reported in the literature. Thus, this study aimed to develop a simple and specific HPLC method for the quality control of GA content in gamboge resin and further apply it for the assessment of GA content in pharmaceutical formulations.

2. Experimental

2.1. Materials
Gambogenic acid, purity >98% (Chengdu Biopurify Phytochemicals Ltd., China) was employed as received and gamboge resin was obtained from a local drug store, Thailand. HPLC grade Acetonitrile, methanol (RCI Labscan) and orthophosphoric acid (Ajax Finechem) were purchased from CT chemical Ltd., Bangkok, Thailand.

2.2. Instrumentation and chromatographic condition
Agilent 1260 HPLC system with a photodiode array detector (Infinity, USA) and C18 column (150 × 4.6 mm, 5 µm particle size, Dr. Maisch, Germany) were used. The mobile phase composed of 85% acetonitrile and 15% orthophosphoric acid (0.1%). The mobile phase was filtered through 0.45 µm membrane filter before use and sonicated for 20 min. The isocratic elution was performed at 1.5 mL/min flow rate with a 12 min of run time. The column temperature was processed at ambient temperature. The sample injection volume was 20 µl and the detection wavelength was at 360 nm.

2.3. Preparation of standard solutions
The accurately weighed amount of GA (10 mg) was dissolved in methanol at a 1 mg/mL concentration using a volumetric flask. The solution was sonicated for 10 min and adjusted to the volume with methanol. Then, the working standard solution was prepared with phosphate buffer solution pH 7.4, to obtain the final concentration of 5 - 120 µg/mL.

2.4. Method Validation
The validation of HPLC method was performed in accordance with ICH guidelines for specificity, linearity, accuracy, limit of detection (LOD), limit of quantification (LOQ) and precision (intra-day and inter-day variation) [7].

2.5. Preparation of GB sample
The gamboge sample was accurately weighed and dissolved in methanol and mixed well before filtration through the membrane filter (0.45 mm). The solution was further diluted to the appropriate concentration. Three different gamboge samples were used for the determination of GA content.

3. Results and discussion

3.1. Method Validation
Specificity
The chromatogram of GA standard solution is presented in Figure 2a. The retention time of the method was approximately 9 min. This developed method provided a shorter analysis time than the previous method [4].

Linearity
The method demonstrated good linearity with a correlation coefficient ($r^2$) of 0.9998. The slope and intercept were calculated using linear regression analysis. The equation was $y = 11.803x + 3.1421$. The linear relationship was evident between the concentration of analyte and the peak area (Figure 3). The linearity range for GA was 5-120 µg/mL.

Figure 2. HPLC chromatograms of GA in standard solution (25 µg/mL) (a) and gamboge resin (b).

**Figure 3.** Linearity of standard gambogic acid detected at the wavelength 360 nm.

Accuracy
The accuracy was investigated as a standard addition method. The known concentration of gamboge sample was prepared by spiking with 8, 10 and 12 µg/mL of gambogic acid solution. The mixture solutions were analyzed and calculated for recovery (%) and relative standard deviation (% RSD) for each concentration. The validated method was shown to be accurate and % RSD of each level was less than 2%. The results are summarized in the Table 1.

| Concentration (µg/mL) | % Recovery (Mean ± SD) | % RSD |
|-----------------------|------------------------|-------|
| Spiked Sample         | Calculated spiked (Mean ± SD) |       |
| Spiked 8              | 8.100 ± 0.077           | 101.1 ± 0.96 | 0.95 |
| Spiked 10             | 9.828 ± 0.095           | 98.87 ± 0.95 | 0.97 |
| Spiked 12             | 12.344 ± 0.068          | 102.92 ± 0.57 | 0.55 |

Table 1. Recovery data of GA at concentration of 8, 10, 12 µg/mL (n = 3).
Precision
The intra-day precision was obtained by determination of six replicates of fixed concentration of GA within a day. The inter-day precision was examined by analyzing six replicates of the same concentration of GA on different days. The results were described as % RSD and are shown in Table 2. The % RSD was within the limit of the acceptance criteria, which was not more than 2%.

Determination of LOD and LOQ
LOD and LOQ were quantitatively determined in accordance with the standard deviation method. The LOD was calculated using the formula LOD = 3.3 × SD/S, and LOD was calculated using the formula LOQ = 10 × SD / S, while SD is the standard deviation of the response and S is the slope of the calibration curves. The results were found to be 2.069 and 6.271 µg/mL, respectively. Although the LOD and LOQ values in this study were higher than in the previous study [4], it was lower enough for precise and accurate quantification of GA in a gamboge sample.

| Conc. of GA (µg/mL) | Observed Conc. of GA (µg/mL) | Mean ± SD | % RSD | Observed Conc. of GA (µg/mL) | Mean ± SD | % RSD |
|---------------------|-----------------------------|-----------|-------|-----------------------------|-----------|-------|
| Intra-day (n = 6)   |                             |           |       | Inter-day (n = 6)            |           |       |
| 20                  | 21.101                      | 20.947 ± 0.114 | 0.546 | 19.907                      | 19.527 ± 0.198 | 1.012 |
|                     | 21.025                      |            |       |                             |           |       |
|                     | 21.008                      |            |       |                             |           |       |
|                     | 20.889                      |            |       |                             |           |       |
|                     | 20.856                      |            |       |                             |           |       |
|                     | 20.805                      |            |       |                             |           |       |

3.2. Determination of GA in gamboge resin
The GA content was calculated using the regression equation (y = 11.803x + 3.1421) obtained from the standard curve. The amount of GA content was 444.2 (± 0.125) mg/g or 44.42% for the determination of three different gamboge samples. The GA content in gamboge resin was reported previously about 337 mg/g [8] and 257 mg/g [4]. The data showed the content of GA was much higher in the developed HPLC method. The chromatogram of GA in the gamboge raw sample is presented in Figure 2b.

4. Conclusions
This study provided a developed and validated HPLC method for the analysis of GA in gamboge resin. The validation results exhibited that the method was simple, reliable with linearity higher than 0.9998, recovery was within 98.87–102.92%, and the RSD was not more than 2% for precision. Thus, the developed method was acceptable for the quality control of GA in gamboge resin.

Acknowledgements
The researchers are thankful to Faculty of Pharmacy (the Research and Creative Fund), Silpakorn University. The authors would like to thank Thailand Science Research and Innovation (TSRI) and Silpakorn University Research, Innovation and Creativity Administration Office for kind support and facilitation.

References
[1] Siji A and K. B. Rameshkumar 2017 Diversity of Garcinia species in the Western Ghats: phytochemical perspective, JNTBGRI, India. 162-169
[2] Liu Y, Chen Y, Lin L and Li H 2020 Int. J. Nanomedicine 15 10385-99
[3] Zhou Z and Ma J 2019 Exp. Ther. Med. 18(4) 2917-23
[4] Song JZ, Yip YK, Han QB, Qiao CF and Xu HX 2007 J. Sep. Sci. 30(3) 304-9
[5] Hua X, Liang C, Dong L, Qu X and Zhao T 2015 Biomed. Chromatogr. 29(4) 545-51.
[6] Qu W, Li Y, Shi D and Qu F 2015 Se pu 33(2) 152-7
[7] ICH Q2(R1) 2005 Validation of analytical procedures: text and methodology, ICH Harmonised Tripartite Guideline (Chicago, USA)
[8] Khaing EM, Chantadee T, Intaraphairot T and Phaechamud T 2019 Key. Eng. Mater. 819 215-20.