METHOD VALIDATION FOR QUANTITATIVE DETERMINATION OF GALLIC ACID FROM *ACACIA CONCINNA* (WILLD.) D.C.

PATTRA AHMADI PIRSHAHID*, CHIRAMET AURANWIWAT, WIRIYAPORN SUMSAKUL, SINEE SIRICOON, WARAPORN SORNDECH

Expert Center of Innovative Health Food, Thailand Institute of Scientific and Technological Research, Pathumthani 12120, Thailand

*Corresponding author: pattra_a@tistr.or.th

Abstract:

Gallic acid is one of the most antioxidative components found in *Acacia* genus. *Acacia concinna* (Willd.) D.C. belongs to *Acacia* genus and Fabaceae family, this genus has been used as traditional medicine. The development and validation of RP-HPLC method to determine gallic acid from the ethanolic extract of *Acacia concinna*. The chromatographic condition was performed on Symmetry Shield RP18 (4.6 x 250 mm) with ACN and 0.5% H_3PO_4 as mobile phases, flow rate 1.0 mL/min and detection at 272 nm. The quantitative results using external standard, standard gallic acid showed the linear range between 5-50 µg/mL while LOD and LOQ exhibited the values at 0.87 and 2.65 µg/mL, respectively. The accuracy and precision also examined. In the real sample, the ethanolic extract of *A. concinna* consisted of gallic acid 1.042 %w/w.

Keywords: Gallic acid; *Acacia concinna*; Method validation

Introduction

Acacia concinna (Willd.) D.C. is a member of Fabaceae family which found in tropical rainforests area in Southern Asia and locally name as "Som-Poi". This plant has used for traditional medicine to treat the skin deceases (Sekie *et al.*, 1999). In some area, the pods of this plant have been used for washing hair and promoting hair growth (Sekie *et al.*, 1999; Tezuka *et al.*, 2000). The report on phytochemical analysis of this plant found the various groups of secondary metabolites including flavonoids (Tezuka *et al.*, 2000), lactam (Sekine *et al.*, 1989), saponins (Gafur *et al.*, 1997; Kiuchi *et al.*, 1997) and terpenoids (Sekie *et al.*, 1997 and Anjaneyulu *et al.*, 1999). These isolated compounds exhibited the arachidonate 5-lipoxygenase activity and cytotoxicity (Sekie *et al.*, 1999; Tezuka *et al.*, 2000). Gallic acid is of the phenolic compounds has been isolated from the plant in *Acacia* genus (Khalia *et al.*, 1989; Salam *et al.*, 2011) and exhibited interesting biological activity such as antioxidant, anti-inflammatory, antitumor and anti-obesity (Thompson *et al.*, 2013; Kim *et al.*, 2019). In this study, we reported the resulted in the method validation of gallic acid to determine quantitative amount in real sample. The linear range, LOD, LOQ, accuracy and precision of this method also investigated and applied to measure in the ethanolic extract of *A. concinna*.

Materials and Methods

General Experimental Procedure

The RP-HPLC was performed on Water 600 controller, equipped with a Water 486 detector, USA using Symmetry Shield RP18 column (4.6 x 250 mm, 5 µm), injection loop 20 µL, flow

rate 1 mL/min and detection wavelength at 272 nm at 30 °C. A binary gradient elution system consisted of acetonitrile (A) and 0.5% H_3PO_4 (B) and using a gradient program: 0-5 min, 0% A, 5-6 min, 30% A, 6-20, 30% A, 21-30 min, 90% A, 31-40 min, 90% A. The ultrasonic bath was Bandelin Sonorex, Banoelin. The acetonitrile and MeOH were purchased in HPLC grade from RCI Lab Scan, Thailand while phosphoric acid was obtained from Merck, Germany. Gallic acid standard compound was purchased from Sigma, Switzerland.

Plant materials and extraction

The pods of *A. concinna* were collected from Chiang Mai Province in 2014. The pods of *A. concinna* (1.0 Kg) were chopped to small pieces then extracted with hexanes (3L x 1). The solution was filtered, and the residue was extract with 95% EtOH (3L x 6). The removal of solvent under reduce pressure provided the ethanolic extract as a dark brown gum.

Preparation of standard Stock Solution

Accurately weighed 10 mg of standard gallic acid then transferred to a 50 mL volumetric flask and dissolved in MeOH 20 mL. The stock solution was sonicated for 15 minutes and adjusted with MeOH to 50 mL. The stock solution has concentration 200 µg/mL.

Method validation

The method was validated and evaluated as per ICH guidelines.11 The parameters lists were specificity, linearity and range, limit of detection (LOD), limit of quantification (LOQ), precision and accuracy.

Specificity

The method's ability established the specific for the target compounds which showed good separation without the effect from matrix. The specific of the method was carried out by a comparison of retention time of sample with the standard.

Linearity and Range

Preparation of standard solution 5, 10, 15, 20, 25 and 50 µg/mL from stock solution into volumetric flask and adjusted with MeOH. The linearity was plotted from the peak area of standard solution and concentration.

Limit of detection (LOD) and limit of quantitation (LOQ)

The values of LOD and LOQ were calculated from the calibration curve using the formula as LOD = 3.3SD or 3.3δ/S while LOQ = 10SD or 10δ/S

Precision

Precision studies were performed on the analysis of six times of the samples which injected as the same condition as the standard gallic acid. The results were expressed in terms of percent relative standard deviation (% RSD).

Accuracy

Accuracy of this method was investigated the recovery experiments by addition the standard compounds of gallic acid into sample solution**.** All samples were analyzed in triplicate and recovery was calculated**.**

Determination of gallic acid in 95% EtOH extract of A. concinna

Accurately weighed 100 mg of 95**%** EtOH extract of *A. concinna* then transferred to a 100 mL volumetric flask and dissolved in MeOH 20 mL**.** The sample solution was sonicated for 15 minutes and adjusted with MeOH to 50 mL**.**

Results and Discussion

Specificity

The specificity of the method exhibited good separation of the standard gallic acid and sample solution**.** The chromatogram illustrated clear peak and did not affect from the other matrix**.** A standard gallic acid and the extract showed peak at retention time 13**.**2 minutes **(**Figure 1**).**

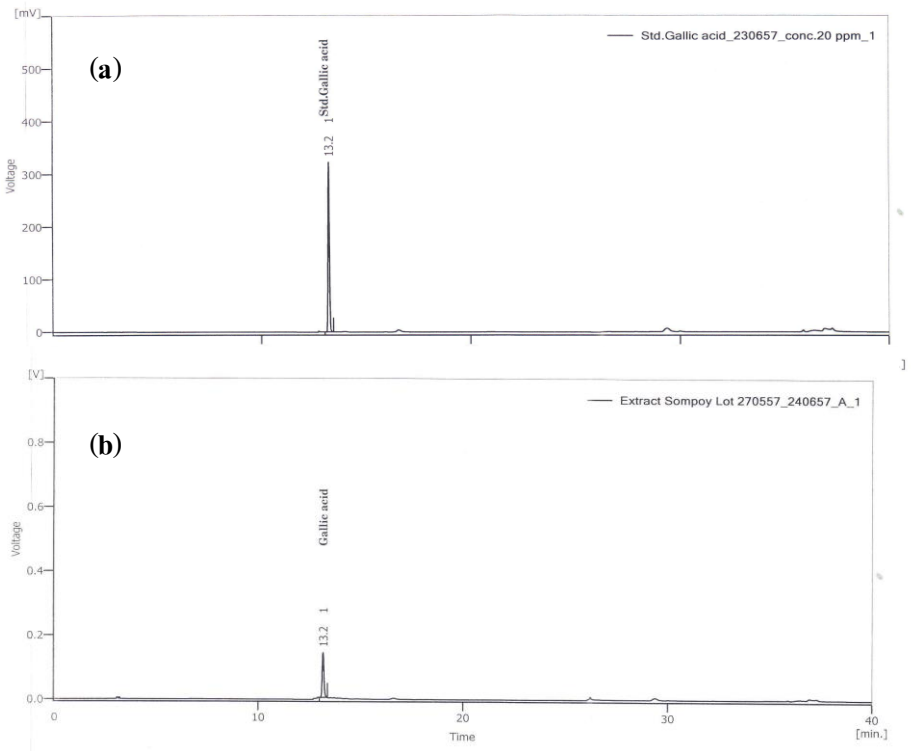

Figure 1: The specificity test of standard gallic acid (**a**) and 95% EtOH extract of *A. concinna* (**b**)

Linearity and Range

The caliblation curve for standard gallic acid presented a good correlation coeffient (R^2 = 0.9998) with the formula y = 72.519x + 33.542 in the scope of concentration between 5-50 µg/mL (Figure 2).

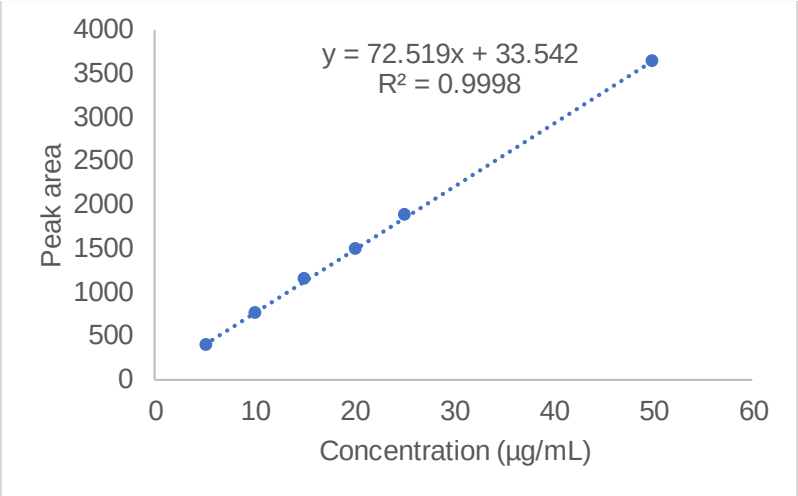

Figure 2: The calibration curve of standard gallic acid

Accuracy and precision

The accuracy tests were performed by the calculation of percent recovery by adding three different concentrations of the standard solution mixed with the real sample. The results showed the percent recovery between 98.667-100.139 % (Table 1). The precision of multiple sampling measurements showed the express of agreement of resulted under prescribed conditions. The relative standard deviation (%RSD) of the measurement established the precision were 0.299-1.754% as shown in Table 1.

Table 1: The summary of method validation data in the tests of accuracy and precision

Sample	%Recovery	%RSD
Std. gallic acid 10 ppm	-	1.754
Sample + std. gallic acid 10 ppm	99.896	0.564
Sample + std. gallic acid 20 ppm	100.139	0.884
Sample + std. gallic acid 30 ppm	98.667	0.299

Determination of gallic acid in 95% EtOH extract of A. concinna

The crude extract of *A. concinna* was measured with the optimized condition and method's validated. A comparison of peak area of standard compound with extract of *A. concinna* found gallic acid contained 10.996 µg/mL or 1.042 %w/w in sample

The method validation of RP-HPLC to determine gallic acid with the optimized condition using Symmetry Shield RP18 column (4.6 x 250 mm, 5 µm), injection loop 20 µL, flow rate 1 mL/min and detection wavelength at 272 nm at 30 °C. Mobile phase were ACN and 0.5% H_3PO_4. This method has been validated in specificity which exhibited good separation in chromatogram without the effect from matrix. The extract and standard gallic acid were eluted at 13.2 minutes supported this peak was gallic acid. Range of this method over 5-50 µg/mL established a good linear relationship between concentration and peak area (R^2 = 0.9998). LOD and LOQ of this method were calculated from the calibration curve found in the concentration of 0.87 and 2.65 µg/mL, respectively. After the standard gallic acid was added in the extract solution, the results showed the acceptable of %recovery in the range of 98.667-

100.139 %. Furthermore, the precision of the method found the %RSD between 0.299-1.754% which suitable for method to measure the quantitation. In the real sample, six ample of 95% EtOH extract solution of *A. concinna* were measured in the aforementioned method. Thus, the extract contained gallic acid in the value of 10.996 µg/mL or 1.042 %w/w from a comparison of peak area with the calibration curve.

Conclusion

The validated method to determine gallic acid in *A. concinna* (Willd.) D.C. has been developed using RP-HPLC. The method is specificity, good linearity and range, accurate and precise to quantitative detection of gallic acid. This phenolic compound is interesting for health industry and this method can apply to search for the other sources of gallic acid in the nature.

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