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Stability Indicating RP-HPLC for Quantification Mangiferin in Extract of Three Species Mango Leaves

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Abstract

The stability indication of Reversed Phase-High Performance Liquid Chromatography (RP-HPLC) method was validated for quantitative determination of mangiferin on three species mango leaves *(Mangifera odorata* Griff, *Mangifera foetida* Lour, and *Mangifera indica* L.*)*. The samples were extracted by maseration method using methanol and concentrated using rotary evaporator. The method carried out on stationary phase a purospher RP-18 endcapped (25 cm × 4.6 mm i.d., 5 µm) column with a mobile phase consisting of methanol: phosphoric acid 0.1% (v/v) (31:69); flow rate:0.8 mL/min; solvent methanol, detection was carried out at 258 nm. The analytical performace this measurement is good with the value of linearity (r^2=0.998), precision (%RSD=0.649%), and accuration (10.67%). The forced degradation studies were carried out according to the International Conference on Harmonization (ICH) guidelines. The results indicating that the complete separation between degradation products and mangiferin peak occured. The degradation limit of mangiferin 5–20% (according to the guideline of ICH) except in basic condition (100%). The method was succesful applied to determine of the mangiferin in pakel (*Mangifera foetida*), kweni (*Mangifera indica*) and kopyor (*Mangifera odorata*) extract. The mangiferin content was obtained are pakel (9.95%), kopyor (7.40%) and kweni (*Mangifera odorata*) (2.49%) respectively.

Keywords: Mangiferin, Mango leaf, Mangifera odorata Griff., Mangifera foetida Lour, Mangifera indica L., RP-HPLC, validation

INTRODUCTION

The Mango was easily found in Indonesia and distributed in all region of Indonesia. Indonesia was one of the country with the highest production of mango (Husen et al., 2012). But, until now only the fruit have been used while the leaves not yet. Mango leaves (Mangifera indica L.) from Anarcadiaceae family contains many chemical compounds such as phenol, β-carotene, flavonoid, tannin, saponin, alkaloid and steroid (Palafox-Carlos et al., 2012 ; Pino et al., 2011). One of the phenolic compounds that found in mango was mangiferin. Mangiferin can be found in all parts of mango plants as peel, pulp, seed kernel (Luo et al., 2012) , bark (García-rivera et al., 2011) and leaves (Jutiviboonsuk et al., 2010). The mangiferin was a phenolic compound that has poten antioxidant activity, and multifactorial pharmacological effects, including antidiabetic, antitumor, lipometabolism regulating, cardioprotective, anti-hyperuricemic, neuroprotective, anti-inflammatory, antipyretic, analgesic, antibacterial, antiviral and immunomodulatory effects (Mirza et al., 2013; Du et al., 2018). Mangiferin is c-glucosyl xanthone and its structures (Figure 1) 2-C-D-glucopyranosyl-1,3,6,7-tetrahydroxy xanthone, fulfill to Lipinski rules (Campa et al., 2012). Based on chemotaxonomy, mangiferin can be found in another mango species; more related the plant so the chemical compound will be more similar (Subha et al., 2007). But, the amount of the compound can be affected by some factors such as the location of cultivation, variety, and stage of maturity.

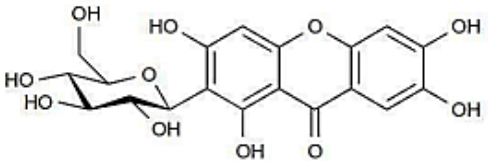


Figure 1. Structure of Mangiferin

Mangiferin was quantified only in one species of mango (Mangifera indica L.) while there are 62 species of mango (Pracaya, 2005). Quantification of mangiferin can be done using High-performance thin layer chromatography (HPTLC) (Subha, et al., 2007),

liquid chromatography-mass spectrometry (LC-MS) (Ilango, et al., 2014) and High-performance liquid chromatography (HPLC) (Zhang, et al., 2014; Eddy, et al., 2014). So, the aim of present study was to develop a stability-indicating RP-HPLC assay method for mangiferin. The developed HPLC assay method was validated as per ICH guidelines Q2 (R1) (Anonim, 2005) and to determine the highest contents of mangiferin in three species of mango leaves so can be used as traditional medicines.

METHODOLOGY

Materials and Instrumentals

Mangiferin standard was purchased from Sigma-Aldrich, India. Methanol used for HPLC was analytical grade and purchased from Sigma-Aldrich, Germany. Water used for HPLC was purchased from WIDA WITMUnicap. Another material used in this analysis were technical methanol, technical phosphoric acid, and membrane filter (0.22 µm). Kweni, pakel and kopyor leaves that were used in this study must had dark green color and taken from the plagiotrop branch and cultivated on January 2019. All the species used had been identified by Faculty of Science University of Jember. The research was carried out at the laboratory for analytical chemistry at Departement of Chemistry Faculty of Pharmacy Jember University.

Methods

Instrumentation and analytical condition

The chromatographic analysis was performed using HPLC Shimadzu Prominence integrated with UV detector, on a purospher® STAR RP-18 end capped with 5 µm particle size. 4.6 mm internal diameter and 250 mm lenght (Merck, Darmstadt, Germany) column with flow rate 0.8 mL/min, wavelength 258 nm, injection volume 20 µL and optimum concentration 10 µg mL^{-1}. Mobile phase consists of methanol: phosphoric acid 0.1% (v/v) (31:69) with isocratic elution technique.

Preparation of mobile phase

The water amount 31 mL of was added by 10% of phosphoric acid (Solution 1). 69 mL of methanol and solution 1 were mixed using Erlenmeyer and filtered through 0.22 µm membrane filter.

Extraction of Kweni, Pakel, and Kopyor leaves

Leaves were washed and dried at room temperature for seven days. The leaves were heated with the oven at 50 ^{o}C for 30 minutes and blend to make the smaller size. Leaves powder weighed 100 mg and extracted by maceration method using methanol 500 mL for 24 hours. Then sample concentrated using rotary evaporator at 50 ^{o}C under pressure (Irmawan, et al., 2018)

Preparation of standard stock solution

The standard stock solution was prepared by weighing 1 mg of mangiferin standard and transferred to 10 mL clean dry volumetric flask. The volume was made up with methanol to obtain 100 µg/mL of mangiferin. The solutions were further diluted with the same solvent to obtain the needed concentration of mangiferin standard.

Preparation of sample solution

The sample solutions were prepared by weighing 1 mg of extract of kweni, pakel and kopyor leaves and transferred to 10 mL clean dry volumetric flask. 4 mL of solvent added and sonicated for 30 min in cold water. Finally, the volume was made up using methanol to obtain 100 µg/mL of the extract kweni leaves. The solutions were further diluted with the same solvent to obtain the concentration of extract kweni leaves 10 µg/mL.

Validation method

Validations method was performed using kweni leaves extract. The method of analysis was validated using recommendation of ICH for parameters like system suitability, linearity, accuracy, precision, detection limit, and quantitation limit.

1. System suitability
   The system suitability was determined by injected standard solution of mangiferin 10 µg/mL six times into system and chromatograms were recorded. The system suitability was determined by %RSD (Relative Standard deviation) of retention time and peak area, theoretical plates and tailing factor.
2. Specificity
   The blank solution, standard solution, and sample solution were injected simultaneously into the system and chromatograms were recorded. Specifity was determined of analyzing the cromatogram of sample in comparison with those obtained for mangiferin standard solution and blank solution aiming at confirming that none of the matrix interfere with the quantitation of the extract.
3. Linearity
   Linearity was determined by a least-square linear regression routine using the compound peak area and concentration of the working standard

solutions prepared at seven concentration levels (6.08; 8.1; 10.1; 12.1; 16.2; 18.2 and 20.2 µg/mL) were prepared from working standard. 20µL each concentrations were independently injected into HPLC system. The linearity of the method was evaluated by calculation of correlation coefficient of calibration curves according to the ICH.

4. The limit of detection (LOD) and limit of quantitation (LOQ)
   The limit of detection (LOD) and limit of quantitation (LOQ) were calculated using following formula: LOD= 3.3(SD)/S and LOQ= 10 (SD)/S, where the SD=standard deviation of response (peak area) and S= average of the slope of the calibration curve (Pangabean, et al., 2016).
5. Intraday and interday precision
   The precision was assessed at intraday and interday time. The intraday precision was determined by measuring kweni leaves extract 100 µg/mL injected six times on the same day. The intermediate (interday) precision was estimated by injecting kweni leaves extract prepared at the same concentrations on three different days. Results were reported in terms of relative standard deviation (RSD) (Napitupulu, et al., 2019).
6. Accuracy
   The accuracy of the method was determined by calculating percentage recovery using standard addition method. The concentration of the standard had been added was 30, 45, and 60% of the concentration of the kweni leaves extract.

Procedure for forced degradation studies

Forced degradation studies were carried out to provide some information about the standard mangiferin and sample solutions stability during analysis. Those solutions were analyzed over a period of 24 h at room temperature. The force degradation studies were conducted by exposing the standard and sample solution with various degradation conditions such as acidic (2 N HCl for 30 min at 60 °C), basic (2 N NaOH for 30 min at 60 °C), neutral (refluxing the extract in water for 6 hours at 60 °C), oxidative (20 % H_2O_2 for 30 min at 60 °C), thermal (105 °C for 6 h), and photolytic (UV chamber for 7 days) (Naim, et. al., 2018, Bandla, et al., 2018).

RESULT AND DISCUSSION

The research indicated that the system suitability parameters were obtained with the mobile phase containing methanol 0.1% (v/v) of phosphoric acid (31:69 % v/v). The mobile phase eluted the extract at retention times 18.939 min. The suitability parameters like resolution, tailing factor, theoretical plate count and % RSD for peak area of five replicate injections of the standard are within limits. The corresponding chromatogram was shown in Figure 2 and the data are presented in Table 1.

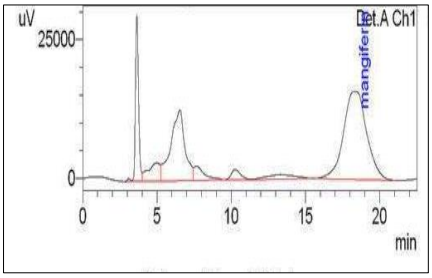


Figure 2. Typical sample chromatogram

The calibration curve for mangiferin content was found to be linear over the range of 6.08-20.20 µg mL^{-1}. The linier regression equation obtained was y = -62737.69 + 88267.09x, where y is the peak area and x is the standard solution concentration. The correlation coefficient (r^2) 0.998171. The data of regression analysis of the calibration curve is shown in Table 2 and Figure 3.

Table 1. System Suitability parameters

No.	Parameters	Mangiferin
1	Tailing factor (T_f)	0.785
2	Resolution (Rs)	9.690
3	Retention time (Rt)	18.939
4	Theoretical plates (N)	1116.367

Table 2. Data of linearity

	Results
Method	Linierity
Probability	95%
Number of data	7
Equation	Y = -62737.69 + 88267.09X
Correlation coefficient	0.9982

The % RSD value were less than 2.0% for all concentrations tested and confirmed the suitable intraday and interday precision of the method. The results obtained for the intraday and interday precision are shown in Table 3. The Rt of mangiferin of standard solution and sample solution are identical. Moreover, the blank solution doesn't produce any peak. Hence the proposed analytical method is specific for estimation of mangiferin. The LOD for mangiferin was found to be 0.544 µg mL^{-} while LOQ was 1.633µg mL^{-}, respectively. The mean recoveries

were found to be 101 to 102.5%. These results demonstrate accuracy for the determination of mangiferin in kweni leaves extract. The accuracy test parameters are summarized in Table 4.

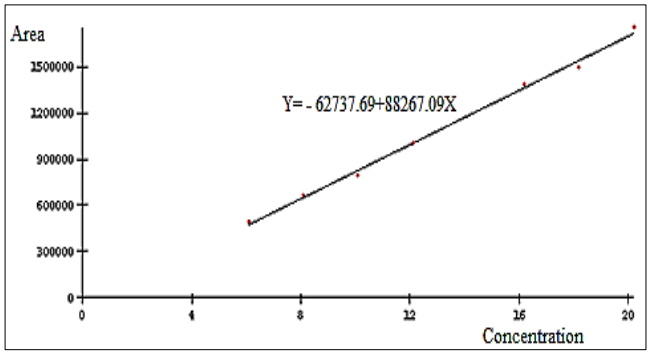


Figure 3. Curve of calibration

The method was continued for testing at the stability of the samples under various stress conditions. Solution of mangiferin standard was exposed toward, acid (2.0 N HCl for 30 min at 60 °C), base (2.0N NaOH for 30 min at 60 °C), oxidizing agent (20% H_2O_2 for 30 min at 60 °C), thermal (105 ^oC for 6 h),UV Light (keeping the standard solution in UV chamber for 7 days). Degradation of drug substances between 5 and 20% has been accepted for validation of chromatographic assays (Taylor et al., 2012).

Table 3. The result of precision test

Precision (% RSD)			
Intraday	Interday*		
n=6	First day	Second day	Third day
0.649%	0.649%	1.295%	1.212%

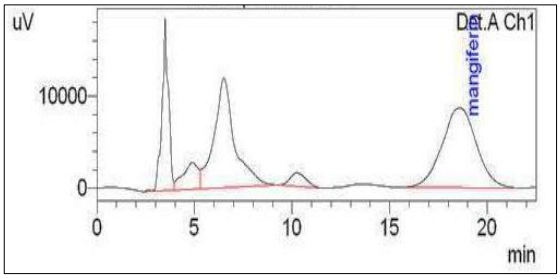
*RSD: Relative Standard Deviation, *Average of six determination*

Table 4. The result of accuracy test

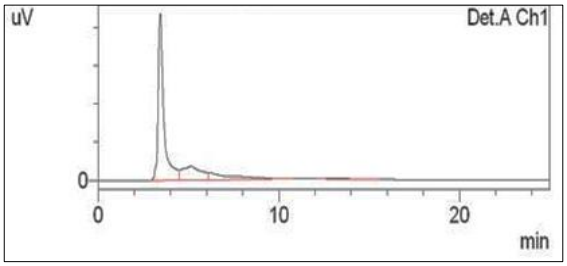
Addition of Standards	*Theoretical Mass (mg)	*Experiment Mass (mg)	#%Recovery	% RSD
30%	0.1652	0.1669	101.0	1.20
45%	0.1770	0.1791	101.2	1.55
60%	0.1982	0.2036	102.8	1.60

**Average of three estimation of extract, #Average of three estimation at each level*

The method was able to detect 18.14 % of decomposition in neutral hydrolysis condition. The chromatograms observed from samples, subjected to various stress conditions, are shown in Figures 4a to 4f. The amount of drug decomposed at various stress conditions are shown in Table 5. From the results, it was found that mangiferin was degraded 100 % in basic condition. In case of acid hydrolysis, the degradation was below the limit for mangiferin. It makes us choosing the pH of buffer in acidic region.



4(a)



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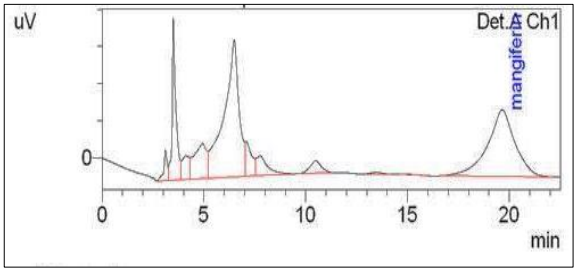

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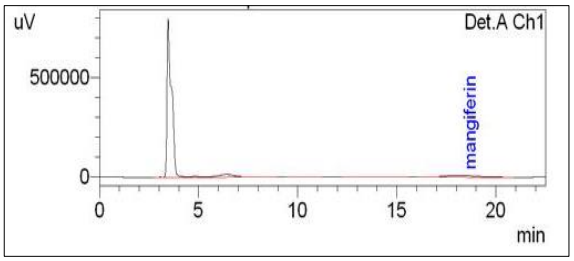

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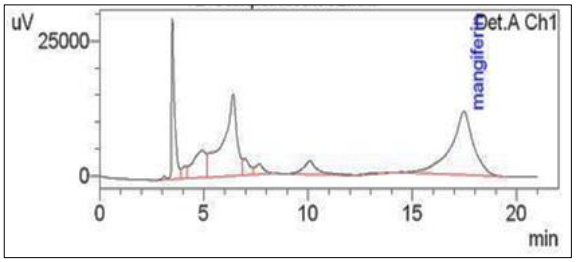

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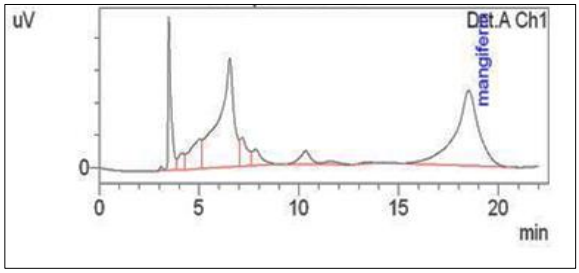

4(f)

Figure 4. HPLC Chromatogram (a) acid degradation, (b) basic degradation, (c) neutral degradation (d) peroxide degradation, (e) thermal degradation, (f) photo degradation

Table 5. Degradation Study of Mangiferin

% Degradation					
Acid hydrolysis	Base hydrolysis	Neutral hydrolysis	Oxidation	Heat	UV
3.79	100	18.14	2.19	11.81	7.90

Table 6. Quantification results of mangiferin in kweni, pakel and kopyor

Samples	*Weight (mg)	*Mass of analyte (mg)	*Content (%w/w)	%RSD
Pakel	5.150	0.5493	9.95	2.13
Kopyor	5.419	0.4014	7.40	1.41
Kweni	5.18	0.1287	2.49	0.65

The next step using the chromatographic analysis is the quantification of mangiferin in kweni, pakel and kopyor. The results of the quantification can be seen in Table 6. The highest concentration of mangiferin found in the pakel leaves extract. We hope there is some research in the future about pakel leaves used as traditional medicines.

CONCLUSION

The proposed stability indicating RP-HPLC method was found to be simple, sensitive, rapid, economical and useful for routine analysis of Mangiferin in the extract of Kweni leaf (*Mangifera odorata* Griff.), Pakel (*Mangifera foetida* Lour.), and Kopyor (*Mangifera indica* L.). The statistical parameters and recovery studies were carried out and reported. The obtained results were satisfactory according to the ICH guidelines. The study showed the leaves of three mango species contain mangiferin with the highest concentration was species pakel.

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