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QUANTITATIVE DENSITOMETRIC ANALYSIS OF FERULIC ACID IN MICROEMULSION OF *ANGELICA SINENSIS* EXTRACT

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INTRODUCTION

Angilica sinensis (Umbeliferae) is commonly used Chinese medicinal herb to enrich blood, promote blood circulation, treat blood deficiency pattern and menstrual disorder such as dysmenorrheal and irregular menstrual cycle (1). Ferulic acid is a major component in the dried roots of *A. sinensis* and has been demonstrated to have a remarkable antioxidant activity (2, 3). It also has been formulated clinically to treat various form of skin trauma and to help wound healing (4). Our researches on *A. sinensis* ethanolic extract focus on formulation of the extract into the microemulsion preparation. This part of study is to develop a densitometric method to quantitative analysis of ferulic acid in microemulsion of *A.sinensis* extract. The method was validated for linearity, precision, accuracy, limit of detection (LOD), and limit of quantitation (LOQ).

MATERIALS AND METHODS

Material and reagents. Reagent and solvents were reagent grade and used without further purification. The dried roots of *A. sisnensis* were purchased from traditional drug stores in Bangkok, Thailand, in May 2012. Ferulic acid was obtained from Aldrich (St. Louis, USA). Methanol was obtained from JT Baker (USA). Spotting device - Linomat 5 automatic sample spotter (CAMAG, Muttenz, Switzerland). Syringe - 100 L (Hamilton, Bonaduz, Switzerland). TLC chamber -Glass twin-trough chamber (20 x 10 cm.) (CAMAG, Switzerland). Densitometer - TLC scanner 3 with winCATS software (CAMAG, Switzerland).TLC plates - 20.0 x 10.0 cm, 0.2 mm layer thickness precoated with silica gel 60 F_{254}, cat. No. 1.05554.0001 (Merck, KGaA, Damstadt, Germany).

Extraction Dried roots of *A. sinensis* (0.5 kg.) were extracted with 95% methanol (1000 mL) in room temperature for 7 days. The extract was filtered through filter paper (Whatman No. 1) and concentrated with rotary evaporator.

Preparation of microemulsion To determine the composition of microemulsions, ternary phase diagrams were constructed. In present study a microemulsion system was prepared by using clove oil as oil phase, tween 20 as surfactant, and distilled water as water phase. Clear and transparent formulations were indicative of a stable microemulsion. Two compositions (oil : tween 20 : water at 2:6:2 , 2:7:1, w/w) were selected for further study.

Preparation of Standard Solution. Stock standard solution was prepared by dissolving 1.2 mg of ferulic acid in 25 mL volumetric flask and adjusted to volume with methanol. Various amounts of the standard solution were spotted on the TLC plate to obtain final concentration at 36, 72, 108, 144, and 180 ng/spot

Preparation of Sample Solution. Microemulsion (oil : tween 20 : water , 2:6:2) of *Angelica sinensis* extract was prepared by adding clove oil (2 g) to *A. sinensis* extract (153 mg) and then added tween 20 (6 g). The mixture was mixed by vortex mixer for a few minutes and the water phase (2 g) was added and mixed by vortex mixer to give a clear solution. An aliquot of the solution (1.0 g) was transferred to 25 mL volumetric flask and dissolved with methanol. For the other microemulsion formula preparation, the procedure was also prepared in the same manner as above by using a different ratio of oil, surfactant, and water.

Validation of the Method. The method was validated for linearity, precision, accuracy, limit of detection (LOD), limit of quantitation (LOQ) according to the ICH (The International Conference of Harmonization guidelines)(5)

(a) *Linearity*-Linearity relationship between peak area and concentration of the standard was evaluated over the range of concentrations expressed in ng/spot by making 5 concentration levels in the rang of 36-180 ng/spot.

(b) *Precision*-Repeatability and intermediated precision of the developed method were expression in term of relative standard deviation (RSD) of peak area. Intra-assay precision studied by repeat analyzing in the same day using concentration (72 ng/spot) of standard solution was applied three times. Interday precision included analysis of the same concentration (72 ng/spot) of solution analyzed for three different days.

(c) *Accuracy*- The accuracy of the method was evaluated by performing recovery studies by adding known amount of the reference compound at three levels (36, 72, and 108 ng/spot) to the sample solutions. Then the solutions were applied on TLC plate and conducted Chromatography. Three determinations were performed for each level of concentration and the recoveries were calculated.

(d) *LOD and LOQ* – LOD and LOQ were determined by scanning the blank (methanol) spot and detected the noise. A series of concentration of ferulic acid standard solution (10-100 ng/spot) were spotted on the TLC palte. Signal-to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ, respectively.

Chromatographic condition of quantitative analysis of ferulic acid in microemulsion of *Angelica sinensis* extract.

Sample was applied to the plate 10 mm. from the bottom and 15 mm. from side edges. Each sample solution (2 μL) was applied in triplicate as narrow band of 6 mm length using a Linomat 5. The constant application rate of 150 nL/s was used. The mobile phase consisted of dichloromethane : methanol : formic acid (98 : 1 : 1, v/v) and the TLC plate was developed using 50 mL of the mobile phase for a plate. The optimized chamber saturation time for the mobile phase was 25 min at room temperature (25 ± 2 C^{o}) The distance covered by the solvent font was 8 cm, which took about 10 min for each run. The spots were scanned using the TLC scanner 3 in the reflectance-absorbance at 254 mm, operated by winCATS software.

RESULTS AND DISCUSSION

The method showed the specificity of separation which ferulic acid had no interference from the matrix (Figure 1). The validation method was composed of linearity, accuracy, precision, LOD and LOQ. For linearity of ferulic acid was determined by the analysis of five different concentration of standard solution, peak areas were found to have good linear relationship with the concentration than peak heights with a correlation coefficient (r^2) of 0.9964 and within the concentration range of 36-180 ng/spot. The correlation coefficient, y-intercept and slope of the regression line of ferulic acid were calculated and present in Table 1. The interday and intraday precission result showed acceptable precision at concentration levels of 72 ng/spot for intraday and interday with RSD < 2 %. The percentage recoveries at three levels of ferulic acid were 99.43, 100.53 and 98.56% respectively (Table 2). When applied to the samples that were prepared with different compositions, the outcomes still showed almost the same result (Table 3).

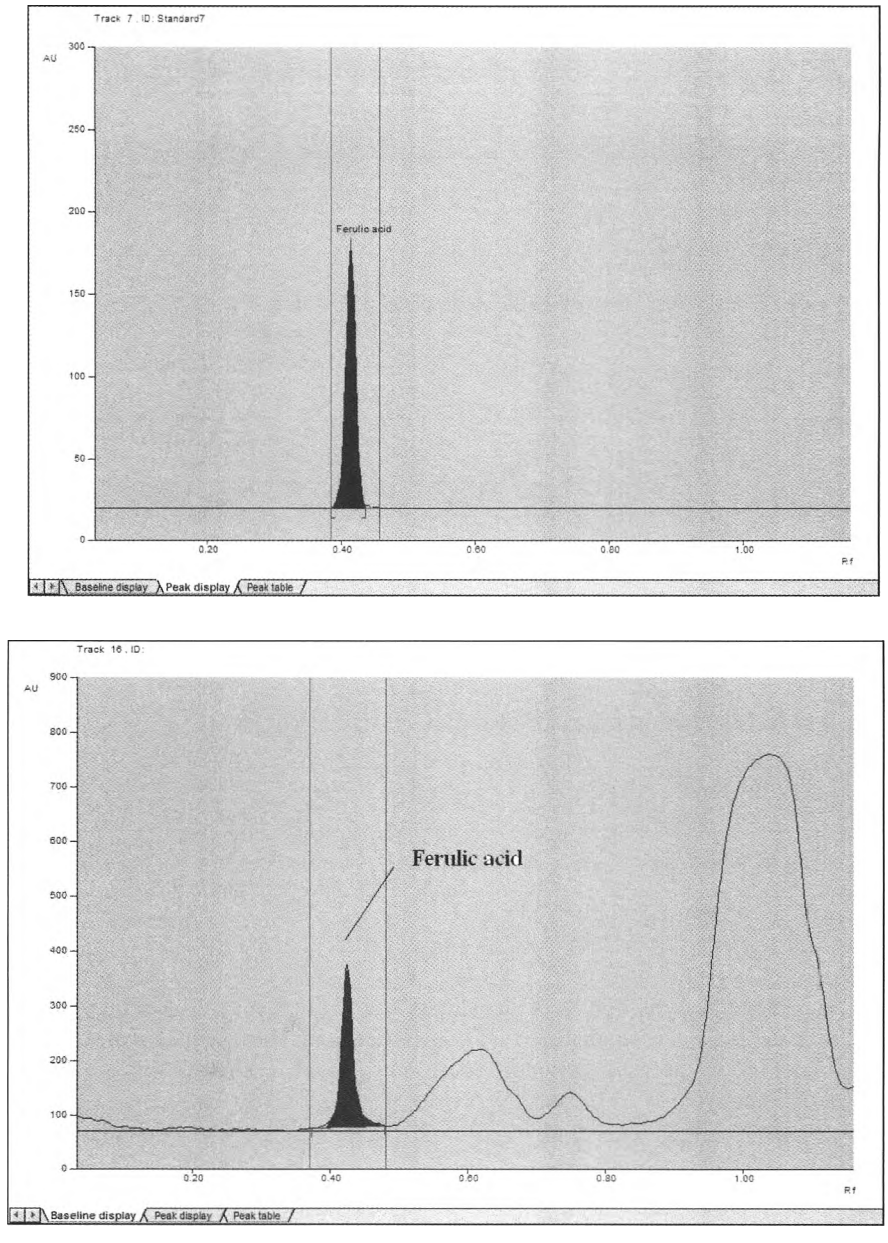

Figure 1. Chromatogram of standard ferulic acid (*above*) and ferulic acid in microemulsion preparation (*below*).

The developed densitometric method was found to be simple, rapid, selective, sensitive and suitable for determination of ferulic acid in microemulsion of *A. sinensis* extract. This method could utilize the merit of applying several sample spots on TLC plate and analyze in a short period of time. In addition, the method was also inexpensive and did not require special types of stationary phase. Thus, it could represent as an alternative method for the HPLC method which needed more time for one sample analysis.

Table 1 Summary of linear regression data for calibration curve of ferulic acid

Parameters	Results
Linear range	36 – 180 ng/spot
Linear regression equation	y = 8.696 + 12.593[a]
Correlation coefficient (r^2)	0.9964
Limit of quantitation, ng	36
Limit of detection, ng	10

[a] x is the amount of ferulic acid in ng, y is the peak area at 254 nm

Table 2 Recovery study of ferulic acid

Serial No	Amount present in the sample, ng	Amount added, ng	Amount found, ng	Recovery[a,b], %
1	86.90	36	122.64 ± 0.71	99.43 ± 3.05
2	95.61	72	167.61 ± 4.55	100.53 ± 1.47
3	86.90	108	202.60 ± 6.91	98.56 ± 0.70

a Experssed as mean standard deviation (SD, n=3)
b Average recovery = 99.51 %

Table 3 Yield of ferulic acid in microemulsion of *A. sisnensis* extract

Formula (oil : surfactant : water)	Yield of ferulic acid, % (w/w) of microemulsion of *A. sisnensis* extract[a]
2 : 6 : 2	1.91 ± 0.02
2 : 7 : 1	2.06 ± 0.03

a Experssed as mean standard deviation (SD, n=3)

CONCLUSION

The densitometric method was developed and validated for qualitative and quantitative analysis of ferulic acid in microemulsion of *A. sinensis* extract. This method was simple, rapid, precise and accurate which could be used for quantitative analysis of ferulic acid in the herb raw material, extract, and herbal product.

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