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# QUANTITATIVE ANALYSIS OF "TEE KA TUK" FORMULATION USING TLC-DENSITOMETRIC METHOD

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**KEYWORDS:** *Piper nigrum*, Black pepper, Piperine, Tee ka tuk, Densitometric method

## INTRODUCTION

Tee ka tuk (TK) is a basic recipe known since ancient time in Thai traditional medicine and it is used to adjust the balance of the elements in rainy season. Its' mechanism of action is explained through the detoxification action which compels the waste out of body particularly through blood and lymphatic system. TK is composed of *Piper nigrum* L, *Piper longum* L (Piperaceae), and *Zingiber officinale* Roscoe (Zingiberaceae) (**1**). *P. ingrum,* commonly known as black pepper, is one of the most important spices in Thailand. It has many pharmacological activities such as vasodilation, anti-inflammatory, anti-gastric ulcers, anti-diarrhea, antibacterial activity and inhibitor acetylcholine esterase (**2, 3**). Black pepper contains 1.0 – 2.5% of volatile oil, and 5 – 9 % of piperine which is a major compound in black pepper. The World Health Organization (WHO) has initiated the need to assure the quality of medicinal plant products by modem control techniques (**4, 5**). We are interested in finding marker compound to do quality control of Thai medicinal formula. This research demonstrated a rapid method for the simultaneous quantification of piperine in the "Tee ka tuk" formulation by TLC-densitometric method.

## MATERIALS AND METHODS

### Material and reagents

Dried fruits of *Piper nigrum* , *Piper longum* and rhizomes of *Zingiber officinale* were purchased from traditional pharmacy in Bangkok Thailand, in January 2011. Reagents and solvents were reagent grade and used without further purification. TLC was performed on silica gel $GF_{254}$ (Merck). For column chromatography, silica gel (Merck 230-400 mesh) was used. NMR spectra were recorded with a Bruker Avance ($^1H$, 300 MHz) spectrometer. Chemical shifts were reported in ppm, and coupling constants were reported in Hz. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$) and referenced to the residual solvent peak. Mass spectra were obtained from Thermo Finigan Polaris Q.

### Isolation and Purification.

Dried powder of fruits of *P. nigrum* 500 g was macerated with 1000 mL of ethanol in erlenmeyer flask for 7 day. Then the extract was filtered and concentrated using rotary evaporator to give a dark brown oil. The crude ethanol extract (18 g) was fractionated by silica gel column chromatography using gradient elution with hexane / ethyl acecate. According to separation, six fractions were collected. The selected fractions were further purified by series of column chromatography and recrystallization to give three compounds.

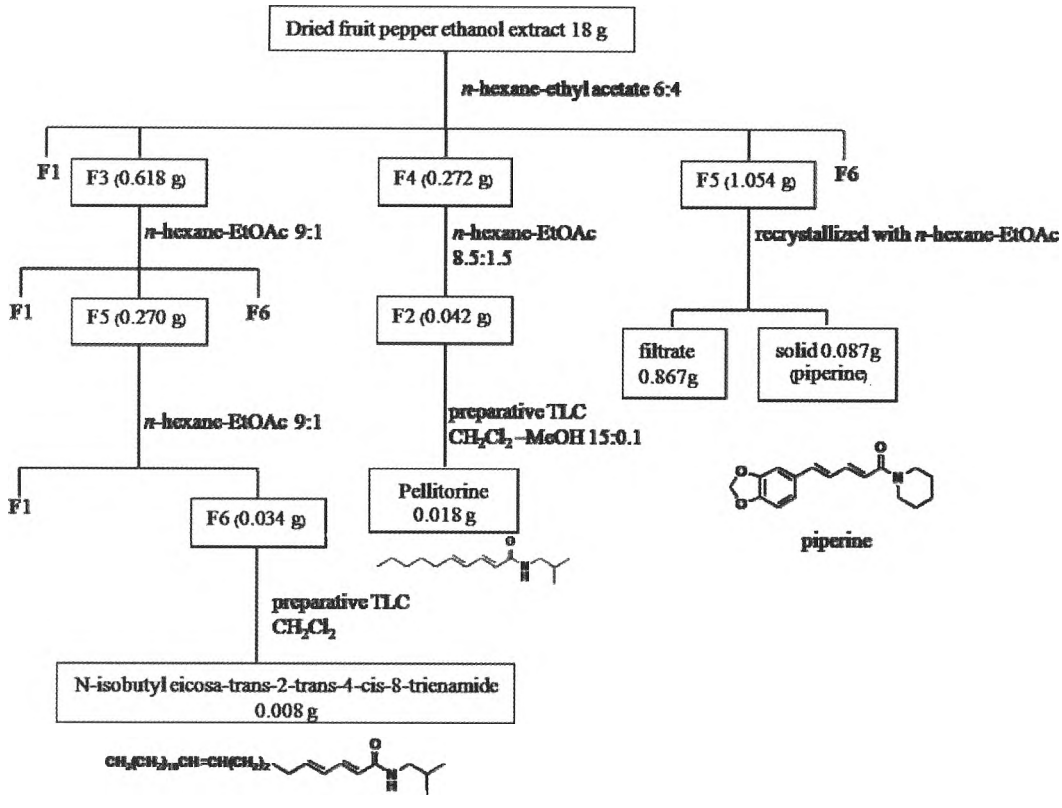
### TLC-densitometric method for estimation of Piperine

### Preparation of standard

A standard solution of piperine (1 mg/mL) 1.0 mL was transferred to a 10 ml volumetric flask. Then methanol was added to making up the volume to yield a concentration of 100 µg/mL of piperine.

### Preparation of sample solution

The formulation sample (3 g) of Tee ka tuk was extracted using maceration process in 50 mL methanol for 3 days. The extract was filtered through a Whatman No.1 membrane and concentrated using rotary evaporator to give dark brown oil. The residue was diluted to volume with methanol to obtain the final concentration of 1 mg/mL




**Scheme 1: Isolation of compounds 1-3 from dried fruits of *Piper nigrum***

**Chromatographic conditions (for piperine)**

| | |
|---|---|
| Stationary phase | : TLC procoated, silica gel G60, $F_{254}$ (Merck) |
| Mobile phase | : Hexane : ethyl acetate (6 : 4 v/v) |
| Applicator | : Linomat 5 (CAMAG, Switzerland) |
| Saturation time | : 15 minutes |
| Detection | : 254 nm (Scanner 3, CAMAG, Switzerland) |

**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 6 concentration levels in the range of 100-600 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 (300 ng/spot) of standard solution was applied three times. Interday precision included analysis of the same concentration (300 ng/spot) of solution analyzed for three different days.

(c) *Accuracy*- The accuracy of the method was evaluated with performing recovery studies by adding known amount of the reference compound at three levels (50, 80, and 100 ng/spot) to the sample solution. 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 piperine standard solution (10-100 ng/spot) were spotted on the TLC plate. Signal-to noise ratios of 3:1 and 10:1 were considered as LOD and LOQ, respectively.

## RESULTS AND DISCUSSION

For column chromatographic separation of ethanol extract of *P. nigrum* fruit, fractions 5 was further purified by column chromatography and recrystallization to give piperine (87 mg) (**6**). Fraction 3 was subjected to column chromatography and preparative thin-layer chromatographic method to obtain N-isobutyl eicosa-trans-2-trans-4-cis-8-trienamide (8 mg) (**7**). For fraction 4, after further purified by column chromatography and preparative TLC, it gave pellitorine (18 mg) (**8**) (Figure 1). All compounds were elucidated by spectroscopic methods and the data were compared with the literatures.

The TLC-densitometric method for quantitative analysis of piperine was validated for linearity, precision, accuracy, LOD and LOQ which showed in Table 1. The calibration equation Y = 346.617 X + 14.210, response for piperine was found to be linear in range of 100-600 ng/spot ($r^2$ = 0.9969). The %RSD of intra-day precision and inter-day precision were less than 2. The accuracy values obtained at three different concentrations (50, 80, and 100 ng/spot) and the recovery were 102.57%. 101.26%, and 100.56%, respectively (Table 2). The average value was 101.5%.

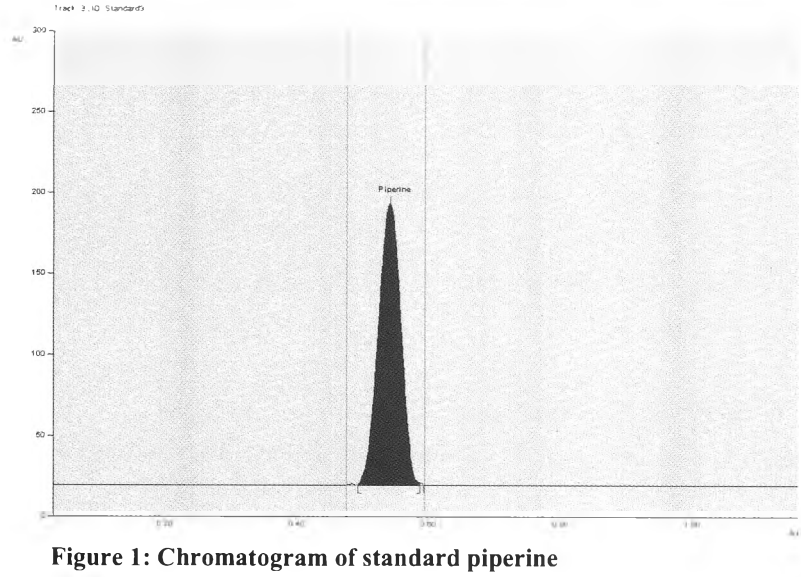



**Figure 1: Chromatogram of standard piperine**

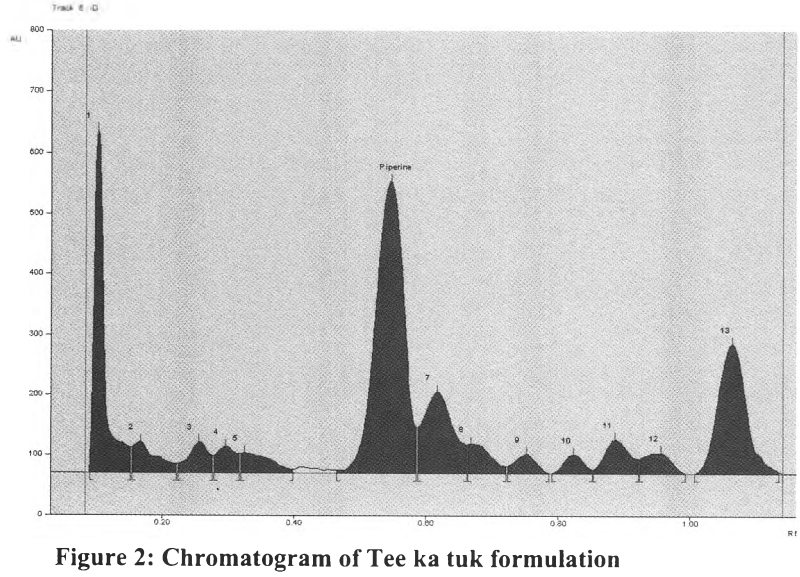



**Figure 2: Chromatogram of Tee ka tuk formulation**

**Table 1 : Validation parameters of piperine**

| No. | parameter | results |
|---|---|---|
| 1 | Range of linearity (μg/ml) | 100.0 – 600.0 |
| 2 | Regression equation | y = 346.617x + 14.210 |
| 3 | Correlation coefficient ($r^2$) | 0.9969 |
| 4 | Precision (intra-day)[a] (%RSD) | 1.05 |
| 5 | Precision (inter-day)[a] (%RSD) | 0.36 |
| 6 | Accuracy | 101.46 ± 1.01 |
| 7 | LOD (ng/spot) | 0.12 |
| 8 | LOQ (ng/spot) | 0.48 |

[a] n = 6

**Table 2 Recovery study[a]**

| Add (ng) | Found (ng) | % Recovery[b] |
|---|---|---|
| 50 | 50.42 ± 1.21 | 102.57 |
| 80 | 81.01 ± 0.87 | 101.26 |
| 100 | 100.56 ± 0.44 | 100.56 |

[a] n = 3

[b] average value = 101.5%.

## CONCLUSION

The proposed TLC-densitometric method was simple, rapid and accurate for quantitative analysis of piperine in Thai traditional formulation extract. It could be used for quantitative analysis of piperine in the single herb extract, raw material, and traditional preparations containing this compound such as "Tee ka tuk" formulation.

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