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Short Communication

Simultaneous Quantification of Sinensetin and Tetramethoxyflavone in Misai Kucing Capsules using TLC-UV Densitometric Technique

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Abstract

Thin-layer chromatographic (TLC)-UV densitometric method is developed for simultaneous quantification of sinensetin and 5-hydroxy--6,7,3', 4'-tetramethoxyflavone (TMF) in Misai kucing capsules (O*rthosiphon stamineus*). Sinensetin and TMF are isolated from the leaves of local O*rthosiphon stamineus.* The TLC-UV desitometric quantification is performed by external standard method on silica gel plates using chloroform-ethyl acetate (60:40) as developing solvent and UV detection at 365 nm.The TLC densitometer, although yields slightly higher values than the other analytical methods, is preferred due to its simplicity, easy and low cost.

Keywords: *Orthosiphon stamineus*; Lamiaceae; Local medicinal plant; HPTLC; Quantification; Densitometer.

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1. Introduction

Sinensetin and TMF are the main flavonoids in the leaves of *Orthosiphon stamineus*, Benth having pharmacological and toxicological activity. They are widely used as a diuretic and to treat rheumatism, diabetes, urinary lithiasis, edema, eruptive fever, influenza, hepatitis, jaundice, biliary lithiasis, and hypertension [1-3]. In particular, extracts of *Orthosiphon stamineus* as a capsules are now widely used in Malaysia as drugs for the treatment of diabetes and kidney stone disease. Polymethoxylated flavonoids have often been examined in the past by spectrophotometric [4] or liquid chromatographic method [5].

Several HPLC methods, mainly based on gradient elution, have been described for their determination in citrus and orange juices. So far no report has appeared on the HPTLC of the polymethoxylated flavones present in Misai kucing capsules. In this paper, we describe a rapid and simple method for the quantitative determination of these two flavonoids in Misai kucing capsules, are presented.

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2. Experimental

2.1. *Chemicals and Reagents*

Methanol, acetone, ethanol and chloroform (analytical-reagent grade) solvents were purchased from Merck (Germany). Water purified by a NonPure-unit (Barnstead, Boston, MA, USA) was used. Standard sinensetin and TMF were isolated and purified by us in our laboratory.

2.2. *Thin layer chromatography (TLC) plates*

Preparative TLC plates (20x10 cm glass plates precoated with thickness 0.05 mm silica gel $GF_{254,}$) were purchased from Merck, Germany. The solvents used to prepare the mobile phase were ethyl acetate and chloroform (analytical-reagent grade) from Merck, Germany. Standards (sinensetin and TMF) and samples were applied to the plates by means of the micro pipette equipped with a 100 µl syringe: the band length was 10 mm; the application volume was 10 µl; the application rates 4 µl/s. Thirteen bands per plates were applied 10 mm from the bottom edge, 15 mm apart. The plate was developed in an unsaturated glass chamber in solvent system chloroform-ethyl acetate (60:40), the migration distance being 8 cm. After separation, the plate was dried in a steam of air for 5 min.

2.3. *Scanning and data processing*

Evaluation of the developed HPTLC plates was performed densitometrically using the CAMAG densitometry analyzer and controlled by an external IBM computer via an RS 232 interface. Data acquisition and processing were performed using the software winCATS.

2.4. *Experimental conditions*

200 mg of the capsule was extracted with 50 mL methanol. The standards such as sinensetin and TMF of concentration 100, 125, 150, 175 and 200 ppm were prepared by standard procedure. The UV-VIS spectra were performed “in-situ” one plate between 200 and 700 nm. The densitograms were obtained at 365 nm in reflection.

3. Results and Discussions

A number of lipophilic flavonoids are present *Orthosiphon stamineus* leaves, sinensetin and TMF being the most abundant. In addition to these two components, orange peel contains nobiletin, 3',4',3,5,6,7,8-heptamethoxyflavone and tangeretin. It should be noted that polymethoxylated flavonoids differ only in the position and the number of methoxy groups. Owing to the hydrophobic nature of these compounds and the small difference in

polarity. Thin layer chromatography (TLC)-densitometry is current method for the quantitation of some polymethoxylated flavonoids in pharmaceutical formulations. Quantitative TLC in situ scanning densitometry is rapidly gaining wide acceptance in pharmaceutical analysis [6-8]. This is because of its simplicity, accuracy, cost effectiveness and the possibility of simultaneous determination of a number of samples on a single TLC plate. The HPTLC allows the identification and the quantification of more than 20 samples in the same chromatographic run. The analysis of the samples requires 15-30 min compared with more than 2 h using a typical HPLC method. Moreover, there is no need for conditioning steps, as with HPLC, and each analysis by HPTLC is less expensive. However, we describe the quantitative determination of sinensetin and TMF from the extract of *Orthosiphon stamineus* as a formulated capsules by using densitometer at 365 nm.

The chromatograms of the samples were visualized in UV light at 365 nm. The chromatograms of the samples show the presence of the spots with same colour and at the same R_f values as the standards.

The quantitative determination was performed by TLC-densitometry using the calibration curve method. Figs. 1a and 1b show the calibration curves obtained for the sinensetin and the TMF, respectively.

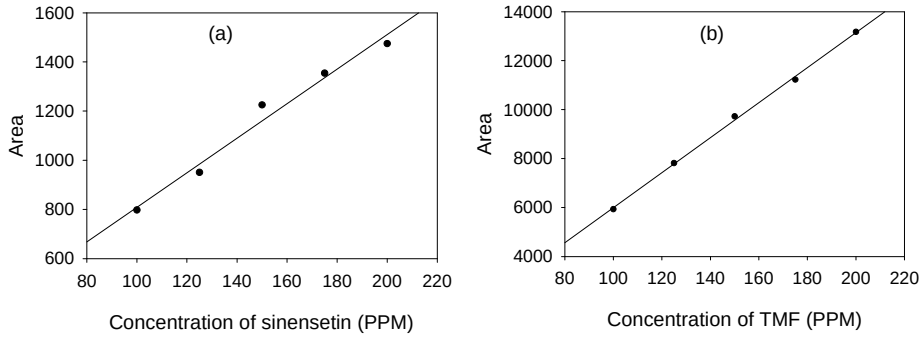

Fig. 1 . Calibration curves for (a) sinensetin, and (b) TMF.

The two curves are represented by the equations:

$y = 7.035x + 104.84$; (R^2=0.9773) (for 1a)

$y = 71.552x$-1163 ; (R^2=0.9985) (for 1b)

where y is the peak area, x is the applied volume.

The concentration was obtained with the formula:

$$C\%\ \text{g/g} = V_e C_{et}/10\text{m}$$

where C% (g/g) is the concentration; V_e is the corresponding volume from the standard,

C_{et} is the concentration of the standard solution, 10 is the quantity of samples in µl , and m is the weight of the plant used for extraction.

Table 1 gives the concentration obtained from the sinensetin and the TMF in the capsules from the extract of *Orthosiphon stamineus* (triplet). It can be observed that the concentration of sinensetin and TMF are almost similar in these two batch capsules.

Table 1. Concentration of sinensetin and TMF was obtained from the samples.

Sample	% yield of sinensetin	% yield of TMF
Capsule 1, 1st Batch	0.26	0.07
Capsule 2, 1st Batch	0.26	0.08
Capsule 3, 1st Batch	0.268	0.07
Capsule 1, 2nd Batch	0.28	0.10
Capsule 2, 2nd Batch	0.28	0.09
Capsule 3, 2nd Batch	0.26	0.10

4. Conclusion

The sinensetin and TMF are determined quantitatively by densitometric method and confirmed by chromatographic and spectral methods. This analytical procedure permits a fast and reliable determination of these drugs in pharmaceutical dosage forms and can be used for routine analysis. However, the scanning densitometry is superior in terms of speed, simplicity and cost.

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References

1. W. Sumaryono, P. Proksch, V. Wray, L. Witte, and T. Hartmann, Planta Med. **57**, 176 (1991). doi:10.1055/s-2006-960060
2. Y. Tezuka, P. Stampoulis, A. H. Banskota, S. Awale, K. Q. Tran, I. Saik, and S. Kadota, Chem. Pharm. Bull. **48** (11), 1711(2000).
3. H. Shibuya, T. Bohgaki, T. Matsubara, M. Watari, K. Ohashi, and I. Kitagawa, Chem. Pharm. Bull. **47**, 695 (1999).
4. L. F. Swift, J. Agric. Food Chem. **15**, 99 (1967). doi:10.1021/jf60149a028
5. R. Roussef and S. V. Ting, in: G. Charalambus, Liquid chromatographic Analysis of Food and Beverage **2**, 537 (1979).
6. D. Agbaba, T. Mijikovic, V. marinkovic, D. Zivanovstakic, and S. Vladimirov, J. AOAC Int. **82**(4), 825 (1999).
7. H. Y. Aboul-Enein and V. Serignse, Anal lett. **27**(4), 723 (1994).
8. B. L. Ling, W. R. G. Baeyens, B. Del Castillo, K. Imai, P. De Moerloose, and K. Stragier, J. Pharm. Biomed. Anal. **7** (12), 1663 (1989). doi:10.1016/0731-7085(89)80180-3