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Short Communication

Quantitative Determination of Sinensetin in *Orthosiphon stamineus* Leaves by Thin-Layer Chromatography and Imaging Densitometry

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Abstract. An analytical method for the determination of sinensetin in *Orthosiphon stamineus* leaves by a thin-layer chromatography-imaging densitometric method was developed. The procedure consisted of extraction of dry leaf powder with 50% methanol and high performance preparative thin layer chromatography (HPTLC). HPTLC was performed on silica gel plate, using chloroform-ethylacetate (60:40) as the developing solvent for sinensetin. The plate was scanned with a reflectance densitometer at 190 nm. The quantification was done by the external standard method.

Keywords: *Orthosiphon stamineus*, sinensetin quantification, sinensetin extraction, reflectance densitometry

Orthosiphon stamineus, family Lamiaceae, is commonly found in the rain forests of several tropical countries. The leaves of this plant are used as diuretic and to treat urinary lithiasis, diabetes, edema, jaundice, hypertension, biliary lithiasis (Hossain and Zhari, 2003), rheumatism (Beaux *et al.*, 1999), and eruptive fever, influenza and hepatitis (Awale *et al.*, 2002). Owing to its pharmaceutical utility, the plant is under systematic cultivation in Malaysia and consumed as a health drink tea to facilitate body detoxification. Extracts of *O. stamineus* are widely used in Malaysia for the treatment of diabetes and kidney stone diseases. Over 4000 chemically unique flavonoids have been identified in plant sources. These low-molecular-mass substances, found in all vascular plants, are phenylbenzopyrones with an assortment of basic structures (Harborne, 1993). On an average, the daily western diet contains approximately 1 g of mixed flavonoids in fruits, vegetables, nuts, seeds, stems, flowers, tea and wine (Kuhnau, 1976). This quantity provides pharmacologically significant amounts of flavonoids in the body fluids and tissues. The most important components of *O. stamineus* leaves are the polyphenols, the polymethoxylated flavonoids, such as sinensetin, eupatorin, and the caffeic acid derivatives, such as rosmarinic and caffeic acids (Olah *et al.*, 2003). The polyphenols from *O. stamineus* have been studied by different chromatographic and spectral methods (Pietta *et al.*, 1991; Wollenweber and Mann, 1985; Gracza and Ruff, 1984). So far, no report has appeared on the HPTLC of sinensetin, which is described as a rapid and simple method for its quantitative determination in the leaves of *O. stamineus.*

Preparative thin layer chromatographic plates (20x10 cm), precoated with l mm silica gel GF_{254} (Merck) were used. The silica gel GF_{254} suspended in water, well homogenized by electric stirring, was applied on the TLC glass plates with a Camag applicator. The tuff was previously sieved and the fraction with particle size < 40 mm was used for the preparation of 0.5 mm thickness wet layer. The water used was purified by the Nonpure-Unit (Barnstead, Boston, USA). The Camag analyser used in the studies was a reflectance spectrometre equipped with an IBM computer, monitoring range 190-700 nm. Data acquisition and processing were performed using the winCATS software programme.

The *O. stamineus* leaves, collected from Penang, Malaysia, were dried at room temperature, or in the oven below 40 °C. The dried leaves were pulverised and sifted through 500 µm mesh size sieve. Accurately weighed 10 g of the leaf powder was transferred to 70 ml 50% methanol (Merck). After ultrasonication for 1 h in an ultrasonic cleaning bath, the extract was filtered and evaporated to dryness (later referred to as extract powder) using rotary evaporator and vacuum pump. Solutions for the standard calibration curve were made in methanol at the concentrations of 0.01, 0.0125, 0.015, 0.0175, and 0.2 mg/ml, using pure crystalline sinensetin. The standard curve was used for the determination of sinensetin in the extract powder. The sinensetin solution of the 50% methanol extract was prepared by dissolving 5 mg extract powder/ml methanol and used for quantitative determination.

The standard sinensetin and the extract powder solutions were applied to the TLC plates (solution volume applied: 10 µl; spot diameter: 10 mm). The solution application was done at 15 mm from the bottom edge of the plate and the spots were 20.1 mm apart from each other. The TLC plates were developed at room temperature in an unsaturated glass twin-trough chamber. The solvent system consisted of chloroform (Merck):

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ethylacetate (60:40), allowing the ascending migration of the mobile phase over a distance of 8.5 cm. The developed TLC plates were dried in a stream of warm air. The chromatograms were read under UV light and further scanned with spectrodensitometre.

The plates were not sprayed with any reagent and the spots were visualised under UV at 365 nm. The chromatograms showed that the spots of the *O. stamineus* extract had their colour and R_f values similar to those of the standard in sinensetin solutions (Fig.1). The calculated R_f values and densitometry for the standards and the components in the extract powder from the leaf extracts of *O. stamineus* further demonstrated the presence of sinensetin (Table 1).

The quantitative determination of sinensetin was done byTLC densitometry using the calibration curve method. The calibration curve already prepared with known concentrations of sinensetin as detailead above, was read using the winCATS software programme. The concentration of the extract powder was obtained using the formula:

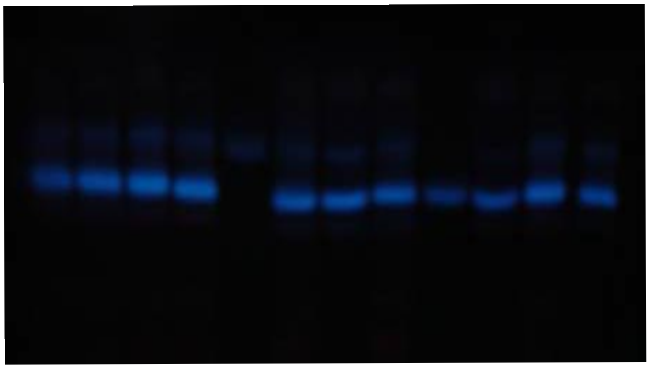

Fig. 1. Chromatograms of the sinensetin extract of *Orthosiphon stamineus* and the sinensetin standard at 365 nm without any reagent sprayed.

Table 1. The concentration of the sinensetin standard solutions and the leaf extract of *Orthosiphon stamineus* applied to TLC plates, their R_f values and the area under reflectance densitometre scanning at 190 nm

Samples /standard	Concentration	Rf values	Area
Standard 1	100 ppm (10 µl)	0.42	790
Standard 2	125 ppm (10 µl)	0.42	902
Standard 3	150 ppm (10 µl)	0.42	1234
Standard 4	175 ppm (10 µl)	0.42	1370
Standard 5	200 ppm (10 µl)	0.42	1532
50% Methanol extract	10 µl	0.42	3784*

* means of three values

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

where:

V_e = volume of the standard solution
C_{et} = concentration of the standard solution
10 = quantity of the extract sample in µl
m = weight of the plant material used for extraction

The amount of sinensetin in the extract powder, calculated from the standard curve by the densitometric method was determined to be 0.36%. The results obtained with this method were found to be in agreement with the confirmatory determinations done on HPLC. The observations on the qualitative and quantitative determinations obtained by the densitometric method were in conformity with chromatographic and spectral methods. The analytical procedure reported here thus provides a fast and reliable method for the determination of sinensetin having the potential of application to other pharmaceutical preparations for the qualitative and quantitative determination of drugs.

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