DETERMINATION OF SWERTIAMARIN CONTENT BY TLC-DENSITOMETER IN FAGRAEA FRAGRANS ROXB. LEAVES

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Abstract: Fagraea fragrans Roxb. (Gentianaceae) is one of the most common herbs in Thai traditional medicine. Various parts of the plant have been used for the treatment of malaria, asthma, fever, and skin eruption. The major component of F. fragrans leaves is swertiamarin, a secoiridoid glycoside. Chromatographic separation of swertiamarin in ethanolic extract was performed on silica gel 60 F254 TLC plates, using the mobile phase of dichloromethane : methanol (10:2, v/v). Detection of swertiamarin was done by densitometric scanning at 240 nm. The identity of the swertiamarin band in the sample extract was confirmed by overlapping the UV absorption spectrum of the sample with that of the reference standard swertiamarin. The linearity range of swertiamarin by TLC-densitometry was 0.3-1.8 µg/spot, (R2) of 0.9995. The precisions calculated by the %RSD of repeatability and intermediate precision, were 0.95 and 1.94 respectively. The average recovery was 101.3. LOD and LOQ were 9.71 ng/band and 29.44 ng/band respectively.

Keywords: Fagraea fragrans Roxb., swertiamarin, method validation, TLC-densitometer.

INTRODUCTION

Fagraea fragrans Roxb. (Gentianaceae) is commonly known as Tembusu and Kankrao in Thailand. The plant is widely distributed throughout Burma to Indo-Malaysia and Thailand. Leaves of F. fragrans are simple, opposite, and elliptic with a thin leathery blade. F. fragrans trees possess small flowers with white petals that gradually turn yellow on fading. Different parts of the plant are used for the treatment of many infectious diseases, such as fungal and bacterial diseases (Pripdeevech and Saansoomchai, 2013). Swertiamarin is a secoiridoid glycoside distributed among the members of Gentianaceae and is reported to have antihyperlipidaemic, hypoglycemic, insulinotropic and antinoceptive activities (Jonville et al., 2008; Singh et al., 2008; Kaikaew et al., 2010; Vaidya et al., 2012; Ahamad et al., 2013).
A thorough literature survey has revealed that few analytical methods have been reported for the estimation of swertiamarin in the plant, its extracts and formulations (Anwar et al., 1996; Alam et al., 2009). However, there are no studies quantifying the swertiamarin content of F. fragrans leaves. The known analytical methods for the estimation of swertiamarin are high-performance liquid chromatography (HPLC), which require user expertise and expensive instrumentation. The TLC-densitometric method has been considered as a simple, rapid, and convenient quantitation method with good accuracy and precision for bioactive components in herbal and crude drugs (Ketmongkhonsit et al., 2015).

The aim of our research is to develop a rapid TLC-densitometric method for simple quantification of swertiamarin in F. fragrans leaves.

![Figure 1. Structures of swertiamarin](image)

**MATERIALS AND METHODS**

**Plant Materials**

*F. fragrans* leaves were collected from Pathum Thani Province, Thailand, in January 2016. The plant was authenticated by Nirun Vipunnguen, Faculty of Pharmacy, Rangsit University, Thailand. The plants were dried at 50 ºC for 24h in a hot air oven and were reduced to coarse powders using a grinder.

**Methods**

**Preparation of plant extract**

Dried *F. fragrans* (0.5g) were sonicated in 5 mL ethanol for 20 min followed by centrifugation. The supernatant was transferred to a 10-mL volumetric flask. The procedure was repeated four times and the extracts were combined. The final volume was adjusted to 10 mL with ethanol. All samples were analyzed immediately after extraction in order to avoid possible chemical degradation. All assays of samples were performed in triplicate.

**Standard solution**

Standard swertiamarin stock solutions were prepared in ethanol and subsequently diluted to obtain a series of standards, ranging from 0.3 to 1.8 µg/spot to construct a calibration curve.
**TLC-densitometric method**

Chromatography was performed on pre-coated silica gel 60 F\(_{254}\) plates 20×10 cm (Merck, Darmstadt, Germany). Samples were applied with a 100 µL syringe using the Linomat V system (Camag, Muttenz, Switzerland). Sample solution was applied as 7 mm bands with a 5 mm distance between the bands. Chromatography was developed in a presaturated state for 30 min in a vertical twin trough glass chamber (Camag, Muttenz, Switzerland), using dichloromethane and methanol (10:2 v/v) as mobile phases. After development, the plate was dried at room temperature for 10 min. Swertiamarin was quantified by direct densitometric scanning of a developed plate at 240 nm and operated by CAT 4 software.

**Method validation**

Various amounts of the standard swertiamarin (0.3-1.8 µg/mL) were analyzed by the TLC-densitometric method as described above and calibration curves were made by plotting peak areas against concentration. The repeatability of the scanning method was tested by replicating the standard swertiamarin (3 concentration x 3 replicates) after application to a TLC plate; then the relative standard deviation percentage (% RSD) was calculated. The variability of the method was studied by analyzing aliquots of different concentrations of standard solutions of swertiamarin (0.3, 0.9 and 1.8 µg/spot) on the same day (intraday-precision) and on different days (interday-precision) and % RSD values were calculated. Accuracy was evaluated by means of recovery assays carried out by adding known amounts of the reference compounds to the sample solutions. Robustness of the methods was determined by small changes in the mobile phase proportions (dichloromethane and methanol (10:2, v/v), (9:3, v/v), (11:1, v/v)). Each experiment was performed in triplicate. To obtain estimates of LOD and LOQ, a series of concentrations of swertiamarin were spotted on TLC plates. LOD and LOQ were calculated by standard deviation of y-intercept of calibration curve.

**RESULTS AND DISCUSSION**

Thin layer chromatography using a densitometer for quantitative analysis of swertiamarin was validated in terms of accuracy, precision, LOD, LOQ and linearity. After the TLC plate was developed, the presence of swertiamarin peak was clearly observed by TLC-densitometric methods in the TLC chromatograms of samples with an Rf value of 0.40 (Figure. 2). The densitograms of swertiamarin under λ max of 240 nm are shown in Figure 3.
Figure 2. (A) TLC photo-documentation at 254 nm (lanes 1-6 were standard, swertiamarin, and lanes 7 - 15 were ethanol extract of \textit{F. fragrans} leaves. (B) TLC-chromatograms of standard swertiamarin and (C) TLC-chromatograms of ethanol extract of \textit{F. fragrans} leaves.

Figure 3. Three dimensional image of the TLC-densitometry scan.
The standard curve of TLC-densitometric method was found by the regression coefficient ($R^2$) of 0.9995 (Figure 4). The repeatability was determined by analyzing the sample solution (3 concentrations x 3 replicates) on the same day, the intermediate precision was determined in 3 different days (Table 1). The LOD and LOQ of TLC-densitometric method were calculated by standard deviation of y-intercept of calibration curve and were found to be 9.71 ng/band and 29.44 ng/band, respectively. Recovery was determined to evaluate the accuracy of the method by spiking known concentrations of standard swertiamarin (0.3, 0.9, 1.8 µg/mL) into F. fragrans extracts with the results presented in Table 1. The average recovery values was 101.3. Swertiamarin contents were 0.606±0.037 % by weight.

Table 1. Validation data of TLC-densitometric method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>TLC-densitometric method</th>
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<tbody>
<tr>
<td>Linear equations</td>
<td>$y = 2.5107x - 7.3933$</td>
</tr>
<tr>
<td>$R^2$</td>
<td>0.9995</td>
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<tr>
<td>Precision (%RSD)</td>
<td></td>
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<tr>
<td>Repeatability</td>
<td>0.95</td>
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<td>Intermediate precision</td>
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<tr>
<td>LOD</td>
<td>9.71 ng/band</td>
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<tr>
<td>LOQ</td>
<td>29.44 ng/band</td>
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<tr>
<td>Accuracy (%Recovery)</td>
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<tr>
<td>0.3</td>
<td>99.7</td>
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<tr>
<td>0.9</td>
<td>102.8</td>
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<tr>
<td>1.8</td>
<td>101.5</td>
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<tr>
<td>Average recovery</td>
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<td>Robustness</td>
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</table>
CONCLUSION

The determination of swertiamarin in *F. fragrans* leaves by TLC-densitometer is a technical analysis method that is simple, rapid, and convenient.

REFERENCES


