Simultaneous determination of physcion and chrysophanol in *Senna tora* seed extract by thin layer chromatography (TLC) – densitometric method

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**Introduction**

*Senna tora* (L.) Roxb. is commonly called Chumhet Thai in Thailand. *S. tora* is one of the medicinal plants in the Fabaceae family and included in Thai Herbal pharmacopoeia volume III¹. *S. tora* is one of well-known anthraquinone plants and have been used in Thai traditional medicines. The seeds were used as laxative and diuretic in the form of decoction. *S. tora* seeds constitute valuable remedy in hyperlipidemia, hypertension, hepatoprotection and skin disease²-³. Extraction of *S. tora* seeds presented antifungal properties including *Trichophyton rubrum*, *T. mentagrophytes*, *Microsporum canis*, *M. gypseum*, *Botrytis cineria*, *Erysiphe graminis* and *Rhizoctonia solani*⁴-⁵. Anthraquinone glycosides are the major constituents of *S. tora* among which physcion and chrysophanol were found to be the active glycosides present as major constituents. Thin-layer chromatography (TLC)–densitometry is significant to chromatographers used for determination of chemical constituents in medicinal plants, herbal products and current drugs because of its simple, rapid and inexpensive. The objectives of the present study to develop a simple, accurate by TLC–densitometric method for the simultaneous determination of physcion and chrysophanol in *S. tora* seed extract and to validate the method.

**Methods**

**Chemical and reagent**

Physcion (purity 99%, HPLC grade) was purchased from Chengdu Biopurify Phytochemicals, China. Chrysophanol was purchased from Sigma-aldrich Chem, USA. Methanol, hexane, ethyl acetate were purchased from Avantor Performance Materials, USA. Acetic acid was purchased from Carlo Erba Reagents, Spain. Ethanol was purchased from Samchai Chemical, Thailand.

**Plant material**

Seeds of *S. tora* from herbal drug store were authenticated by Professor Nijsiri Ruangrungsi, Ph.D. Plant samples were cleaned and dried by hot air oven at 60 degree Celsius for 12 hours. Milling and sieving by sieve no. 60 then stored in tight container and protect from light. The 10 grams of dried powders of *S. tora* seed powders were extracted with 500 ml of 80% ethanol by sonication method for 60 minutes. The extracts were filtered through Whatman no.1 filter paper, solvent evaporated by rotary evaporator and dried by water bath.

**Preparation of sample solution**

Three hundred milligrams of *S. tora* seed extract were dissolved in 10 ml of methanol. This solution was kept in refrigerator at 4-8°C for analysis by TLC-densitometry.
Preparation of standard solution
The stock solution of physcion and chrysophanol (2 mg/ml) were prepared with methanol. These standard solutions were diluted to 0.5 mg/ml with methanol and kept in refrigerator at 5-8°C.

TLC-densitometry condition
This study using stationary phase as TLC plate that pre-coated with silica gel 60 F_{254} (Merck, Germany) and mobile phase is mixing solvent compose of hexane: ethyl acetate: acetic acid, in a ratio of, 7.5: 2.5: 0.1 v/v/v. The substance solutions were applied on TLC plate by automatic spotter model Linomat 5 (CAMAG, Switzerland) with 100 µL of glass syringe (Hamilton, Switzerland). After spotted, the sample plates were dipped in TLC tank and dried at room temperature. After that the developed plats were scanned by densitometer model TLC Scanner 4 which linked to winCATS software (CAMAG, Switzerland) for qualitative and quantitative analysis.

Method validation of TLC-densitometry

Specificity
The specificity of quantitative analysis of physcion and chrysophanol in S. tora seed extract were determined by comparing with retention factor (Rt) and UV-absorption spectra of sample spots to that of standard physcion and chrysophanol using TLC scanner and winCATS software.

Linearity
Linearity was determined by using physcion and chrysophanol standard solution of 0.5 mg/ml in methanol (n=3). The 1.5, 2.0, 3.0, 4.0 and 8.0 µl of physcion, 2.0, 3.0, 4.0, 5.0 and 10.0 µl of chrysophanol standard solution were applied on TLC plate corresponding to concentrations of 750 – 4,000 ng/spot and 1,000 - 5,000 ng/spot for physcion and chrysophanol, respectively. The calibration curve was obtained by plotting the peak area against concentration of standard solution. The slope and intercept values were calculated by linear regression equation.

Precision
The precision of quantitative analysis of physcion and chrysophanol in S. tora seed extract were determined by repeatability (intraday) and intermediate precision (interday). Both precisions were performed by analyzing sample solution of 3 level concentrations (each one in triplicate) on the same day and three different days, respectively. The percentage relative standard deviation (%RSD) was calculated by following formula (1):

\[ \% \text{RSD} = \left( \frac{\text{SD}}{\text{Mean}} \right) \times 100 \]  

Accuracy
The accuracy was tested by standard addition method. The physcion and chrysophanol standard was added directly to the samples. Three different volumes (2, 3 and 4 ml) of the standard solution (0.5 mg/ml) were added to the sample solution (30 mg/ml) and completely mixed. Then all solutions were analyzed by TLC-densitometry. The recovery and average recovery were determined by using following formula (2):

\[ \% \text{Recovery} = \left( \frac{A - B}{C} \right) \times 100 \]  

Where,  
A = Test amount of reference standard in spiked sample extract.  
B = Test amount of reference standard in unspiked sample extract.  
C = Amount of reference standard.

Limit of detection (LOD) and Limit of quantitation (LOQ)
LOD and LOQ were determined from calibration curve of linearity by using following formula (3), (4):

\[ \text{LOD} = 3.3 \times (\text{SD} / \text{slope}) \]  

\[ \text{LOQ} = 10 \times (\text{SD} / \text{slope}) \]

Quantitative analysis of physcion and chrysophanol in S. tora seed extract
The 8.0 µl of S. tora crude extract (30 mg/ml) was spotted on TLC plate by Linomat 5 in triplicate. The sample plates were dipped in TLC tank with developing solvent until to solvent-front position. The sample plates were detected by TLC scanner for quantitative analysis and densitometric scanning at 289 and 258 nm to detect physcion and chrysophanol, respectively. The contents of physcion and chrysophanol were calculated based on the calibration curve of peak area and concentration of standard compounds.
Results

The extraction yield of *S. tora* seed was 18.3% (w/w) of dry powder. The validation data of TLC-densitometric method were shown in Table 1. The *R*<sub>f</sub> value was 0.65 for physcion while the chrysophanol was 0.72. The maximum absorption wavelength was 289 and 258 nm for physcion and chrysophanol, respectively. These result as shown in Figure 1 and 2. The linear equation of physcion was \( y = 4.2656x + 601.49 \) and \( R^2 = 0.9999 \) in range 750 – 4,000 ng/spot, while the chrysophanol was \( y = 1.2496x + 1594.6 \) and \( R^2 = 0.9990 \) in range 1,000 – 5,000 ng/spot. The %RSD values of intraday and interday precision were 5.58% and 7.96% for physcion, 2.96% and 5.37% for chrysophanol. The average recoveries at three levels were 99.47 and 91.16 for physcion and chrysophanol, respectively. The LOD and LOQ of both compound were calculated by calibration curve were 152 and 460 ng/spot for physcion, 258 and 781 ng/spot for chrysophanol. The content of physcion in ethanolic crude extract of *S. tora* seed is 3.48±0.01 % w/w of crude extract, while the chrysophanol is 4.49±0.02 % w/w of crude extract.

Table 1 Validation data of TLC-densitometric method

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Physcion</th>
<th>Chrysophanol</th>
</tr>
</thead>
<tbody>
<tr>
<td>Range (ng/spot)</td>
<td>750-4,000</td>
<td>1,000-5,000</td>
</tr>
<tr>
<td>Linear equation</td>
<td>( y = 4.2656x + 601.49 )</td>
<td>( y = 1.2496x + 1594.6 )</td>
</tr>
<tr>
<td>( R^2 )</td>
<td>0.9999</td>
<td>0.9990</td>
</tr>
<tr>
<td>Precision (%RSD)</td>
<td></td>
<td></td>
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<tr>
<td>Intra-day</td>
<td>5.58</td>
<td>2.92</td>
</tr>
<tr>
<td>Inter-day</td>
<td>7.96</td>
<td>3.31</td>
</tr>
<tr>
<td>Accuracy (%Recovery)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1,000 ng/spot</td>
<td>102.29</td>
<td>91.21</td>
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<tr>
<td>1,500 ng/spot</td>
<td>92.55</td>
<td>94.49</td>
</tr>
<tr>
<td>2,000 ng/spot</td>
<td>103.53</td>
<td>88.07</td>
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<tr>
<td>Average recovery</td>
<td>99.47</td>
<td>91.16</td>
</tr>
<tr>
<td>LOD (ng/spot)</td>
<td>152</td>
<td>258</td>
</tr>
<tr>
<td>LOQ (ng/spot)</td>
<td>460</td>
<td>781</td>
</tr>
</tbody>
</table>

![Figure1](https://example.com/figure1.png)

Figure 1. TLC finger prints of (a) physcion, (b) chrysophanol and (c) *S. tora* seed extract.

![Figure2](https://example.com/figure2.png)

Figure 2. Overlay UV-absorption spectra of (a) physcion standard and *S. tora* seed extract, (b) chrysophanol standard and *S. tora* seed extract.
Discussion
The extraction method in this study was adapted from the study of anthraquinones extraction from *Rheum palmatum* L. This method could be used to extraction of anthraquinones from *S. tora* as well as *R. palmatum* L. The advantage of this extraction method is easy, rapid and safe solvent. The mobile phase in this study was previously used for identification of *S. tora* seed extract but the *R* value of phycion and chrysophanol was not reported in THP. The validation test is followed by ICH guildeline.

Conclusion
TLC-densitometric method showed satisfactory validation and could be determined the content of phycion (3.48±0.01%) and chrysophanol (4.49±0.02%) in *S. tora* seed extract. TLC is extensively used because of its simple, rapid and inexpensive analytical method for chemical analysis. The combination of TLC plate and densitometer was adapted to qualitative and quantitative determination of chemical compound in medicinal plants, herbal products and current drugs.

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References