Development of a New Densitometric-TLC Method for Determination of Asiaticoside Content in Centella asiatica

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Asiaticoside (Fig. 1), a major active compound in Centella asiatica L., is an ursane-type triterpene glycoside with no particular chromophore for UV spectrophotometric analysis. In this study, derivatization of asiaticoside with 2-naphthol on a TLC silica gel plate was developed for direct determination of the triterpenoid glycoside in C. asiatica crude extracts using the wavelength of UV-visible range. The developed densitometric TLC appeared to be simple, accurate and rapid.

In practice, crude extracts of C. asiatica are prepared from the plant materials under reflux with 80% methanol and followed by a step of solvent extraction using dichloromethane and butanol. The partially purified extract is then separated on a silica gel 60 F_{254}, 0.25 mm thickness. The TLC plate was then developed into the solution of 2-naphthol sulfuric acid reagent to obtain a brownish band of the glycoside. After the 2-naphthol derivatization, the plate is scanned under the wavelength of 530 nm to obtain the TLC chromatograms (Fig. 3,4) of various samples. This new method has good sensitivity and selectivity, with the linear range for the analysis from 100-1000 ng/band (Fig. 5) (r^2 = 0.99) and good precision and accuracy (1.15-1.9 %RSD, 98.18-104.3%).

**TLC-Densitometric analysis.** Crude extracts of C. asiatica samples (10 μL each) and asiaticoside standard solution (2 μL) were spotted onto a pre-coated siliga gel (Siliga gel 60 F 254, 0.25 mm thickness). The TLC plate was then developed for 9-10 cm. from the origin using the solvent system of chloroform : methanol : DI water (30:15:1.2). The plate was dried, dipped into 2-naphthyl sulfuric acid reagent, then heated with TLC plate heater at 120ºC for 5 minutes for completing reaction. After the derivatization, the TLC plate was scanned under the wavelength of 530nm. The predicted reaction product of the derivatization is shown in Fig. 6.

**In conclusion,** we have shown a simple, accurate, method of TLC-densitometric analysis of asiaticoside. This new method consists of 2 steps. First, asiaticoside within the crude extracts is separated from other components on a siliga gel plate by a normal thin layer chromatography. Second, the band of asiaticoside on the plate is derivatized with 2-naphthol acid reagent to add the chromophore of 2-naphthol to its structure which can be quantitated directly from its TLC-densitometry chromatogram (λ=350). The method showed good sensitivity and selectivity and appears to be comparable to the UV-HPLC method which is standard method.

*Fig. 3 TLC-patterns of some C. asiatica crude extracts observed under visible light before (A) and after (B) derivatization with 2-naphthol acid reagent.*

*Fig. 4 TLC-densitometric chromatograms of some C. asiatica crude extracts by using solvent system of chloroform-methanol-water: 30:15:1.2.*

*Fig. 5 Calibration curve of standard asiaticoside obtained using the new TLC-densitometric method.*