Rapid and Sensitive TLC-Densitometric Analysis of Artemisinin in *Artemisia annua* L.

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Although artemisinin absorbs light in the ultraviolet region between 210 and 220 nm, its extinction coefficient is poor. As a result, the methodology of standard UV detection is ineffective in the quantitative analysis of artemisinin. The detection of artemisinin has, therefore, been accomplished by using pre-column derivatization to convert artemisinin into a UV active compound that absorbs with a large extinction coefficient at longer wavelengths which are applicable to HPLC-UV method. To overcome this complication, we developed a simple TLC-densitometric technique for rapid and accurate analysis of artemisinin in a large number of *Artemisia annua* plantlets. This new analytical method is based on structural conversion of artemisinin on a silica gel plate by ammonia vapor to form a chromophore-containing compound that can be detected by UV-based TLC-densitometry. The compound elucidated to be 10-azadesoxyartemisinin has its λ_{max} at 310 nm. This wavelength was used for analyzing artemisinin content after samples of crude extracts from various *A. annua* plantlets were subjected to artemisinin separation (on a silica gel plate using a mobile phase of hexane : ethylacetate : acetone, 16:1:1), followed by exposing the plate with ammonia vapor in a TLC tank at 100°C for 2 h. Evaluation of the TLC system was carried out in terms of product stability, precision, accuracy and calibration. Good linearity was obtained in the range of 0.01-0.12 mg artemisinin. The technique appeared to be accurate and sensitive as compared with the complicated pre-column reaction-HPLC technique. Among 90 samples of the plantlets, artemisinin content in the leaves appeared to be highly variable, ranging from 0.02 to 0.67 % w/w of dry weight. These results prove that densitometric TLC can be a cheap and simple technique for the accurate screening of high-artemisinin producing plants.

Keywords: TLC-densitometry; quantitative analysis; artemisinin; 10-azadesoxyartemisinin; Artemisia *annua* L.; Asteraceae.

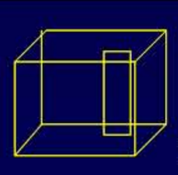
1. Development of a New Analytical Method for Artemisinin Determination

Dried leaves

Reflux with hexane for 4 h

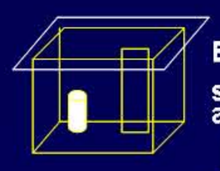
Artemisinin solution

Spotted onto a TLC plate



Developed in solvent system

Hexane:ethylacetate:acteone, 16:1:1



Exposed with saturated ammonia

TLC-densitometric scanner

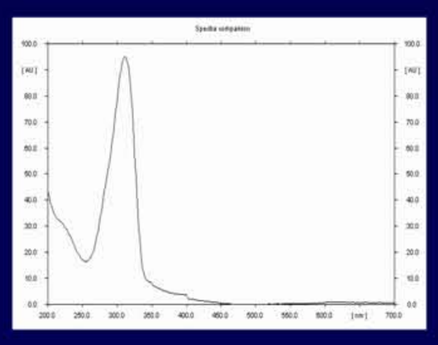


Fig. 1 Absorption spectra obtainted from TLC-densitometric scan of authentic artemisinin and of artemisinin derivative obtained from NH_3-exposure of artemisinin on a TLC-plate at 100°C for 2 h.

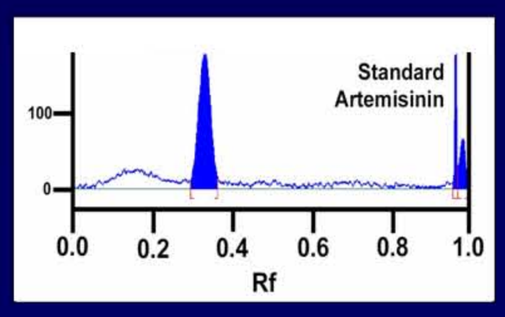
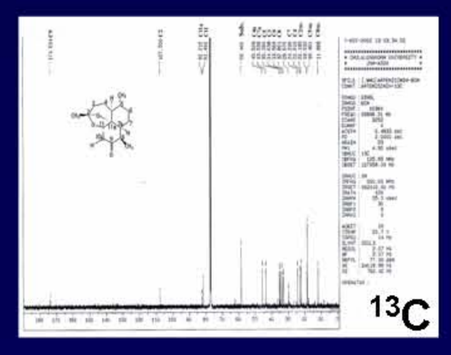


Fig. 2 TLC-densitometric scan of NH_3-derivativatized artemisinin after TLC development of authentic artemisinin using a solvent system of hexane: ethylacetate:acetone, 16:1:1 followed by NH_3-exposure of the plate.



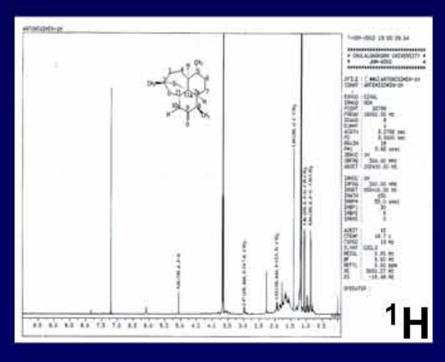


Fig. 3 ^{13}C and ^{1}H-NMR spectra of the derivative of artemisinin after exposing artemisinin on a TLC plate with saturated ammonia at 100°C for 2 h.

TLC-Densitometric analysis. Crude extracts of various A. annnua samples (10 µL each) were spotted onto a pre-coated silica gel plate (Silica gel 60 F254, 0.25 mm thickness). Up to twelve samples were applied onto each standard TLC plate. The plate was then developed using the solvent system of hexane : ethylacetale : acetone (16:1:1) with 8 cm height of solvent front. The plate was dried and exposed for 2 h with saturated ammonia vapor in a closed TLC tank (in a hot air oven at 100° C) for complete chromophore development of artemisinin. The TLC plate was then taken from the tank, air-dried and scanned under the wavelength of 310 nm.

NMR analyses of the product of NH_3-treated artemisinin on silica gel plate. Structural conversion of artemisinin on a silica gel plate after being exposed with NH_3 vapor was analyzed by NMR. The resulting ^{1}H-NMR ($CDCl_3$) δ (ppm),1.93 (1H, ddd, J=13, 4.5, 4.5, CH_2-3), 2.97 (1H, ddd, J=14, 7, 4, CH-8), 6.20 (1H, brs, CH-10), 5.06 (1H, d, J= 3, CH-11), 1.40 (3H, s, CH_3-2), 0.86 (3H, d, J=7, CH_3-5), 1.06(3H, d, J=7, CH_3-8) and ^{13}C-NMR ($CDCl_3$) δ (ppm), 107.5 (C-2), 34.6 (C-3), 22.6 (C-4), 45.6 (C-4a), 35.3 (C-5), 33.6 (C-6), 24.3 (C-7) 43.5 (C-7a), 32.9 (C-8), 173.6 (C-9), 81.5 (C-11), 82.2 (C-11a), 22.2 (CH_3-2), 18.5 (CH_3-5), 11.9 (CH_3-8) were closely similar to those of the compound 10-azadesoxyartemisinin described previously (Torok and Ziffer, 1995).

Proposed mechanism of 10-azadesoxyartemisinin formation from artemisinin

2. Variation of the Artemisinin Content in *A. annua* plantlets

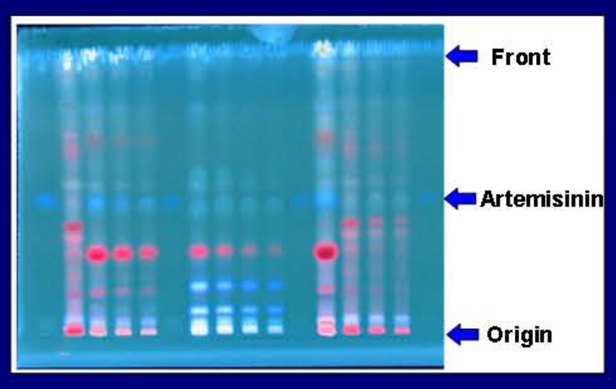
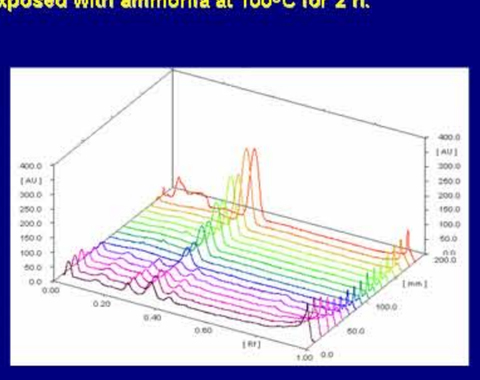


Fig. 4 TLC-patterns of some *A. annua* crude extracts observed under 366 nm after exposed with ammonia at 100°C for 2 h.



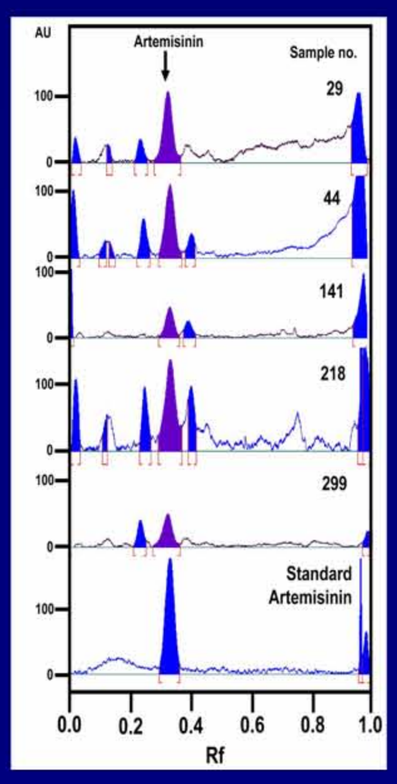


Fig. 5 TLC-densitometric chromatograms of crude extracted from some *A. annua* plantlets using the solvent system of hexane: ethylacetate: acetone, 16:1:1.

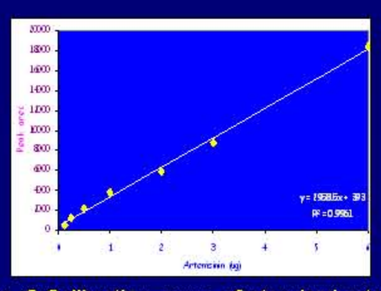


Fig. 6 Calibration curve of standard artemisinin determined by TLC-densitometric method.

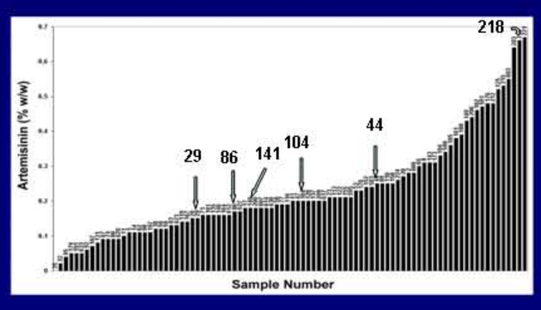


Fig. 7 Artemisinin content in various *A. annua* mutants obtained after the plants were exposed with 500 rad

Conclusion

In conclusion, we have described here a simple, accurate and inexpensive method of TLC-densitometric analysis of artemisinin for rapid screening of high artemisinin-producing plants of *A. annua*. This developed method consists of two main steps. First, artemisinin present in the crude extracts of *A. annua* leaves is separated from other components on a silica gel plate by a normal thin layer chromatography. Second, the spot of artemisinin on the TLC plate is exposed with NH_3 vapor to rearrange its structure to a chromophore-containing compound of 10-azadesoxyartemisinin which can be quantitated directly from its TLC-densitometric chromatogram (λ310 nm). The method appears to be as sensitive and accurate as the HPLC-UV method with precolumn reaction described previously (Zhao, 1986). The simplicity of this TLC-densitometric method obviously allowed us to screen for high-artemisinin producing plants form a large number of *A. annua* plantlets obtained from gamma irradiation. The results showing high variation of artemisinin content in the population obviously indicate the successful application of this analytical technique in the screening of the high-artemisinin yielding plants.

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