## Dihydroartemisinin

The method for a 40 mg dihydroartemisinin and 320 mg piperiquine phosphate combination tablet published in the Minilab manual, Volume II, Supplement 2011, pages 16-19, was modified after checking for the presence of piperaquine phosphate, which is naturally fluorescence quenching under 254 nm UV light. Simply heating the plate caused the dihydroartemisinin spots to be visible in daylight, quench fluorescence at 254 nm, and fluoresce at 366 nm so that use of the sulfuric acid staining method was not necessary for detection. Users may consider that elimination of the detection reagent makes this method safer and more convenient, especially for use in the field.

In the modified method, the exact procedures published in the Minilab manual were carried out with two exceptions. Instead of a 40 mg reference tablet for the standard, 40 mg of commercial analytical grade standard (dihydroartemisinin, Sigma-Aldrich, No. 1001660075) was used. Also, instead of dipping the plate in the methanolic sulfuric acid staining solution followed by heating on a hotplate to detect the dihydroartemisinin as colored spots in daylight and fluorescent spots under 366 nm UV light as shown in the photograph on page 19 of the Minilab manual, the drug was detected as yellow-brown spots in daylight, fluorescence quenching spots under 254 nm UV light, and fluorescent spots under 366 nm UV light, as shown in the photographs of the three plates below, by heating on a hotplate. The 100% working standard solution and 100% working sample solution were 2 mg/mL, and 2 uL volumes were spotted on the plates. The mobile phase was ethyl acetate-methanol-concentrated ammonia solution (16:4:3).

The detection of dihydroartemisinin as fluorescence quenched zones under 254 nm UV light on silica gel glass plates with a fluorescent indicator (F plates) by reagent free thermochemical activation (heating at 160°C for 5 minutes) was first reported in the literature by J. Strock, M. Nguyen, and J. Sherma (Acta Chromatographica, DOI: 10.1556/1326.2016.28.3.6).

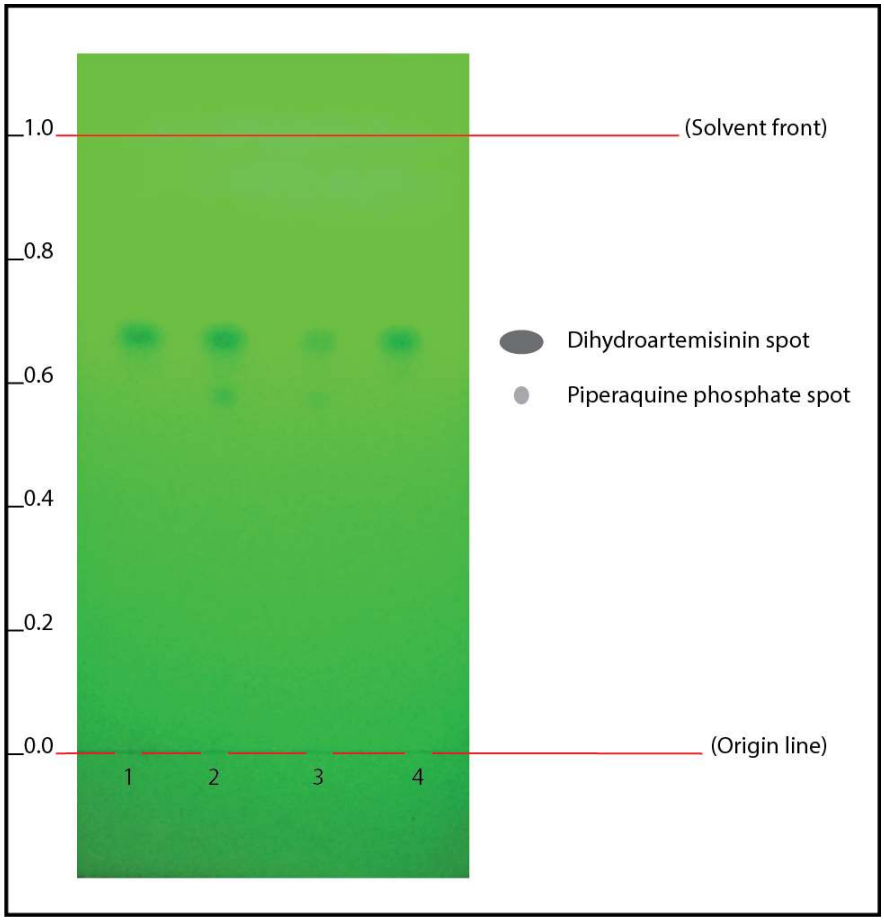
## XI. CHROMATOPLATE OBSERVED UNDER 254 NM UV LIGHT AFTER HEATING

Run No.1:
Upper working standard representing 100% of total anhydrous dihydroartemisinin

Run No.2:
A drug product of good quality with acceptable drug content

Run No.3:
A drug product of poor quality with unacceptable low drug content*

Run No.4:
Lower working standard representing 80% of total anhydrous dihydroartemisinin



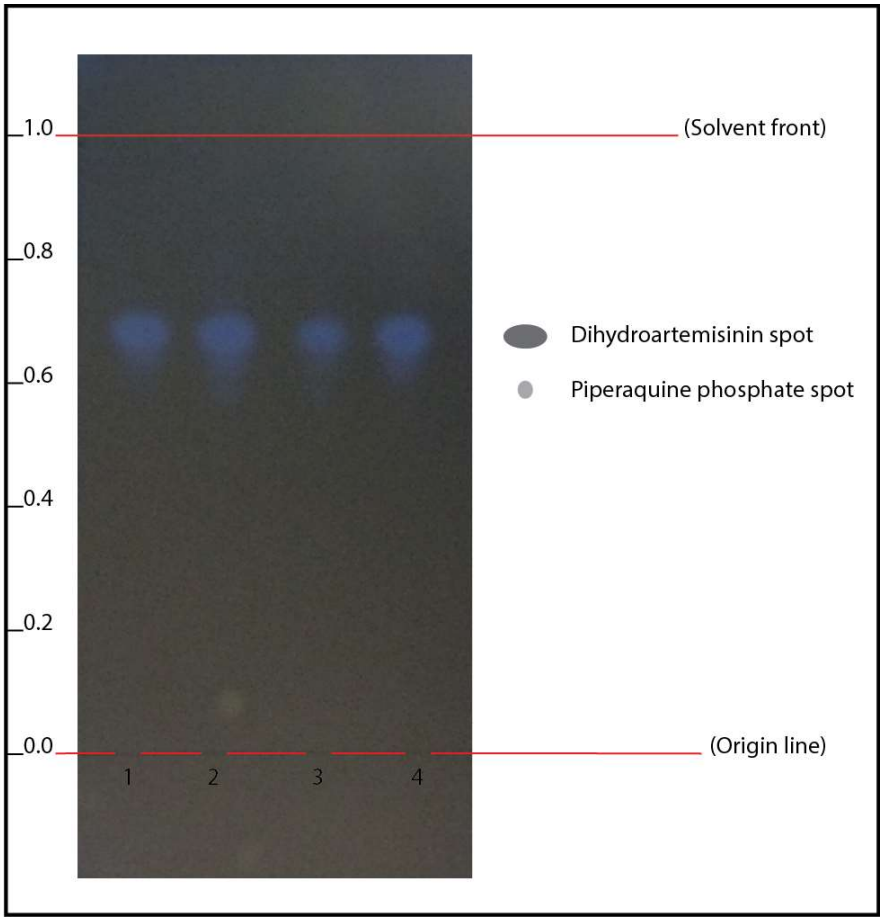

## XI. CHROMATOPLATE OBSERVED UNDER 366 NM UV LIGHT AFTER HEATING

Run No.1:
Upper working standard representing 100% of total anhydrous dihydroartemisinin

Run No.2:
A drug product of good quality with acceptable drug content

Run No.3:
A drug product of poor quality with unacceptable low drug content*

Run No.4:
Lower working standard representing 80% of total anhydrous dihydroartemisinin



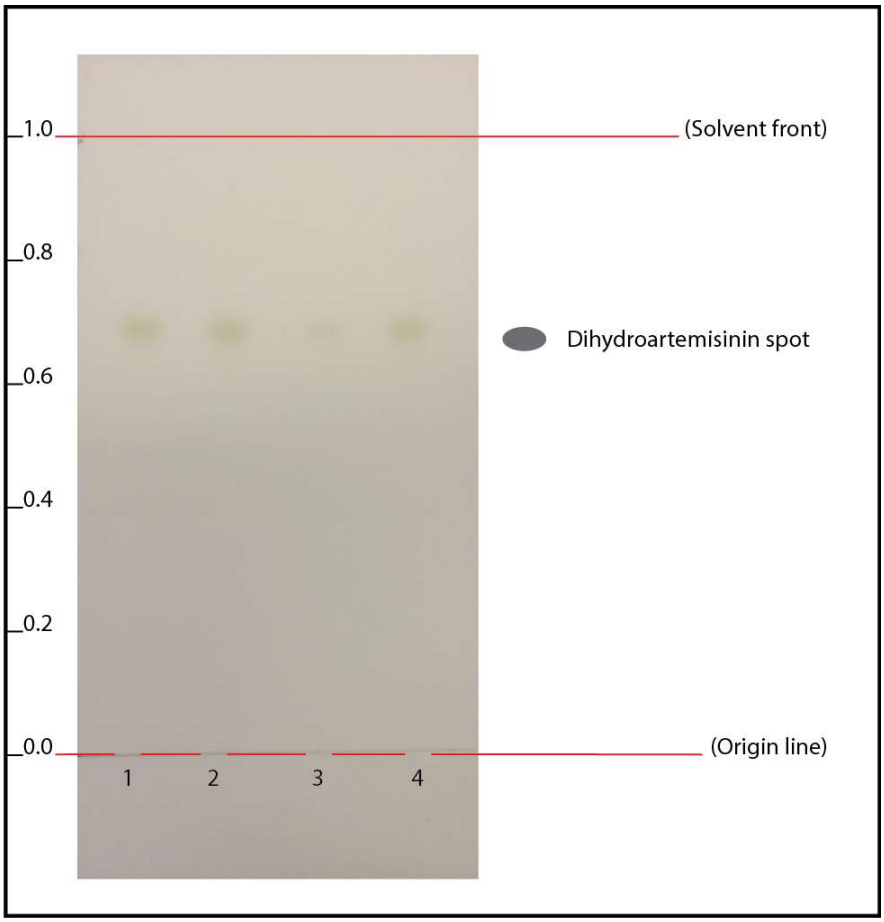
## XI. CHROMATOPLATE OBSERVED IN DAYLIGHT AFTER HEATING

Run No.1:
Upper working standard representing 100% of total anhydrous dihydroartemisinin

Run No.2:
A drug product of good quality with acceptable drug content

Run No.3:
A drug product of poor quality with unacceptable low drug content*

Run No.4:
Lower working standard representing 80% of total anhydrous dihydroartemisinin




(*A drug product of poor quality was simulated by diluting the 100% working sample solution of a drug product of good quality with methanol to one-third of the theoretical value.)

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