

## Clarithromycin

The method for a 250 mg clarithromycin tablet published in the Minilab manual, Volume II, Supplement 2013, pages 16-19, was modified by simple heating of the plate to cause the clarithromycin spots to quench fluorescence at 254 nm and fluoresce at 366 nm so that applications of the sulfuric acid and iodine staining methods were not necessary for detection. Users may consider that elimination of the detection reagents 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 a few exceptions. Instead of a 250 mg reference tablet for the standard, 250 mg of commercial analytical grade standard (clarithromycin, Sigma-Aldrich, No. A3487) was used. Also, instead of exposing the plate to iodine staining or immersing the plate in methanolic sulfuric acid solution followed by heating on a hotplate to detect the clarithromycin as orange-brown or brown-black colored spots, respectively, in daylight as shown in the photograph on page 19 of the Minilab manual, the drug was detected as fluorescence quenching spots under 254 nm UV light and fluorescent spots under 366 nm UV light, as shown in the photographs of the two plates below, by heating on a hotplate. The 100% working standard solution and 100% working sample solution were 12.5 mg/mL, and 2  $\mu$ L volumes were spotted on the plates. The mobile phase was methanol-ethyl acetate-concentrated ammonia hydroxide (20:5:0.5).

The detection of clarithromycin 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 10 minutes) was first used by Ellen Armour and Joseph Sherma for the transfer of the clarithromycin Minilab manual method to a quantitative HPTLC-densitometry method. This research is currently being written up for publication in the Journal of Liquid Chromatography & Related Technologies.

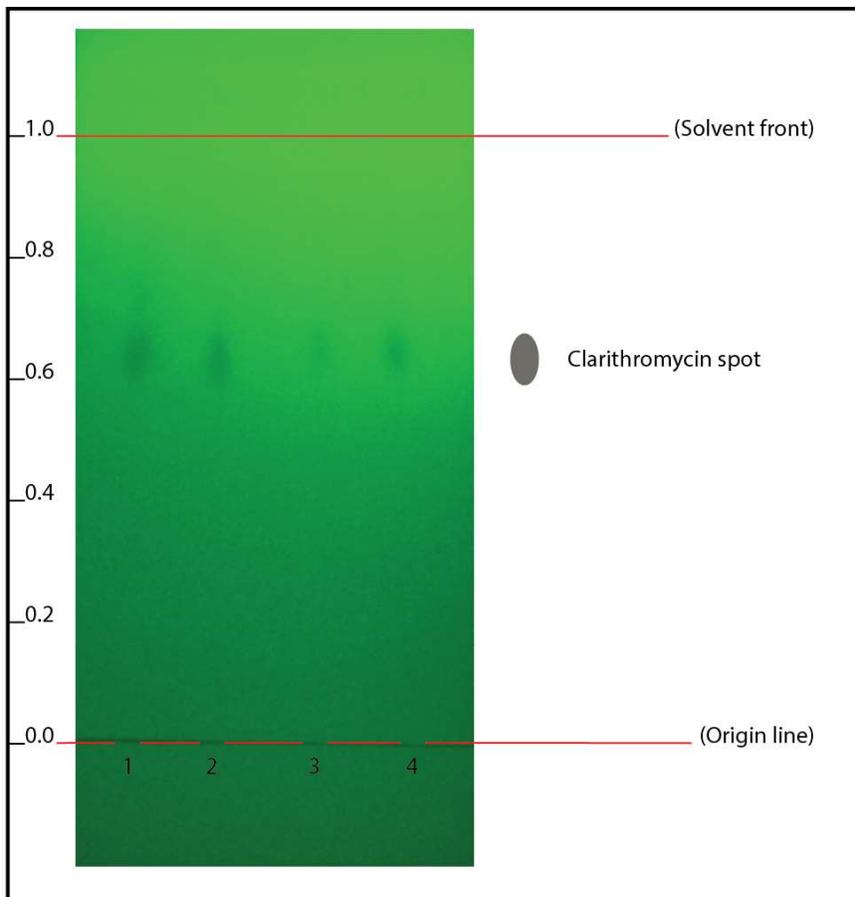
XI. CHROMATOPLATE OBSERVED UNDER 254 NM UV LIGHT AFTER HEATING

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

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 clarithromycin



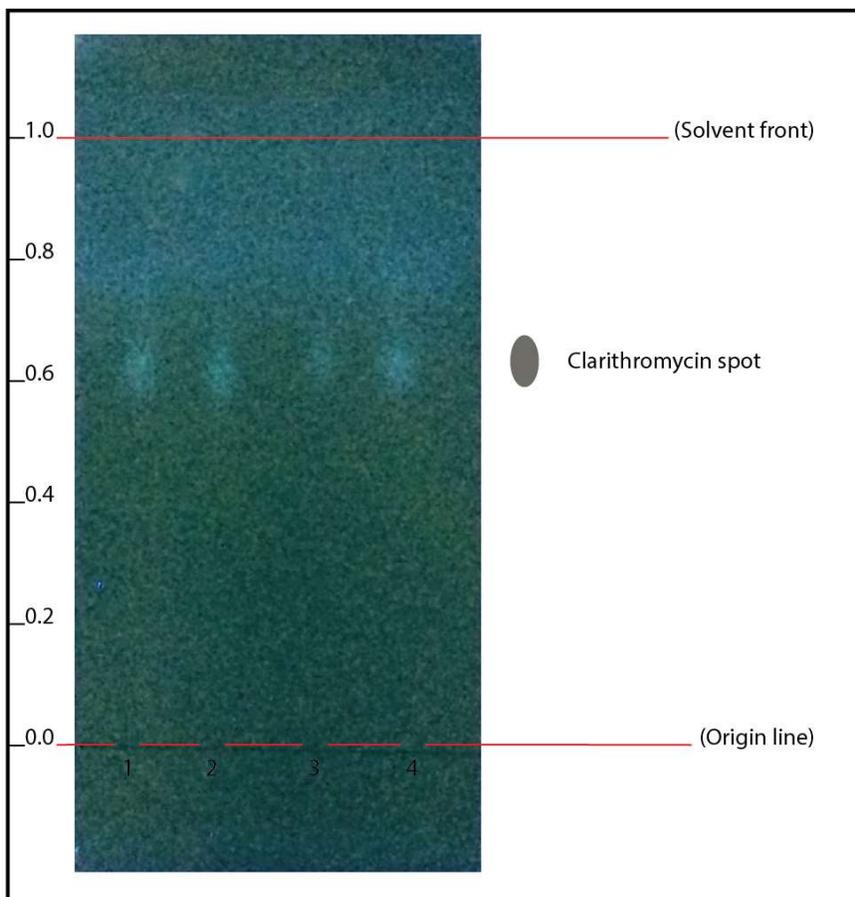
XI. CHROMATOPLATE OBSERVED UNDER 366 NM UV LIGHT AFTER HEATING

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

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 clarithromycin



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

This modified method was developed and tested by Ellen Armour and Joseph Sherma, Department of Chemistry, Lafayette College, Easton, PA, USA. July, 2016. Ellen Armour's EXCEL Scholar research was supported by a Camille and Henry Dreyfus Foundation Senior Scientist Mentor Program award to Professor Sherma.