

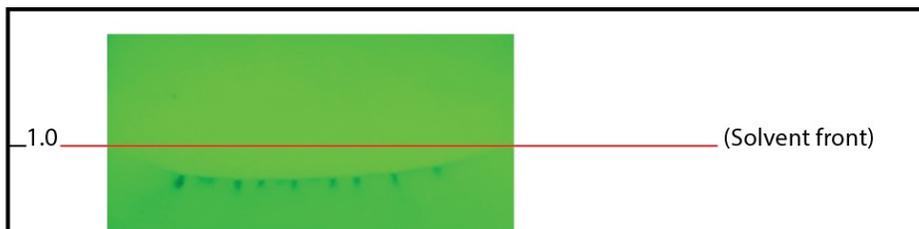
Gentamicin

The method for a 40 mg gentamicin free base per mL ampule published in the Minilab manual, Volume II, Supplement 2016, pages 20-23, was modified by simple heating of the plate to cause the gentamicin spots to be visible in daylight, quench fluorescence at 254 nm, and fluoresce at 366 nm so that applications of the iodine staining method and ninhydrin method were not necessary for detection. Users may consider that elimination of the detection reagents make 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 one exception. Instead of exposing the plate to iodine staining or ninhydrin followed by heating on a hotplate to detect the gentamicin as yellow-orange or red-brown spots, respectively, in daylight as shown in the photograph on page 23 of the Minilab manual, the drug was detected as fluorescence quenching spots under 254 nm UV light, fluorescent spots under 366 nm UV light, and brownish spots in daylight, 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 5.0 mg/mL, and 2 μ L volumes were spotted on the plates. The mobile phase was methanol-water-concentrated ammonium hydroxide (7:3:10).

The detection of gentamicin 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 40 minutes) was first used by Ellen Armour and Joseph Sherma to allow for the transfer the gentamicin Minilab manual method to a quantitative HPTLC-densitometry method (Journal of Liquid Chromatography and Related Technologies, accepted for publication in Spring 2017).

XI. CHROMATOPLATE OBSERVED
UNDER 254 NM UV LIGHT AFTER
HEATING



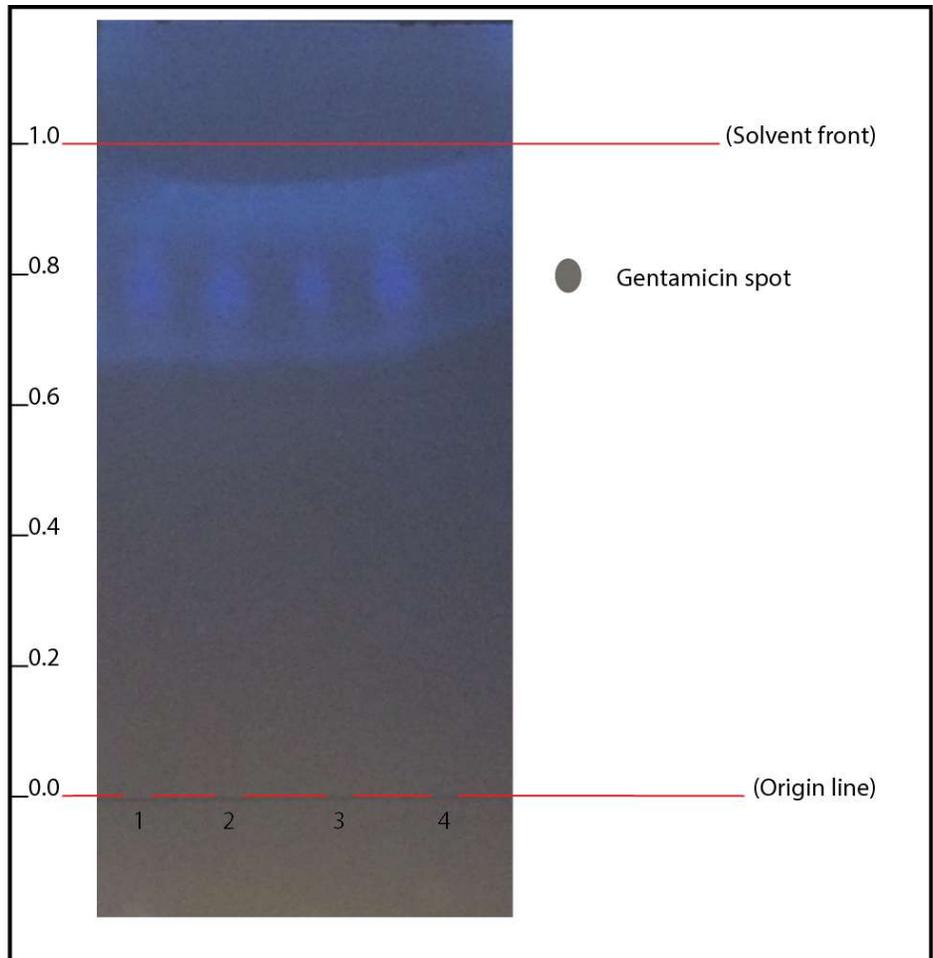
XI. CHROMATOPLATE OBSERVED UNDER 366 NM UV LIGHT AFTER HEATING

Run No.1:
Upper working standard
representing 100% gentamicin

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% gentamicin



IN DARKNESS AFTER HEATING



(*A drug product of poor quality was simulated by diluting the 100% working sample solution of a drug product of good quality with water 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, August, 2016. Ellen Armour's EXCEL Scholar research was supported by a Camille and Henry Dreyfus Foundation Senior Scientist Mentor Program award to Professor Sherma.