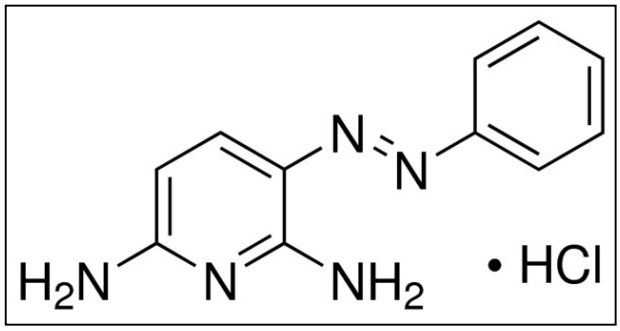
Phenazopyridine HCl
200 mg Tablet

Structure:



Molecular Formula and Mass: $C_{11}H_{11}N_5 \cdot HCl$ – 249.70
Category: urinary tract analgesic
Sample:

Grind one tablet and dissolve in 100 mL methanol. Shake at least 10 min. Concentration of solution = 200 mg/100 mL = 2.00 mg/mL. Solution is then filtered and 1.00 mL is further diluted with an additional 11.0 mL methanol. Final concentration of sample solution = 0.167 mg/mL, which is the required concentration representing 100%.

Standards:

<u>High Standard:</u>

The high limit is 115%; therefore the concentration of the high standard = (0.167 mg/mL X 1.15 = 0.192 mg/mL. Weigh approximately 19.2 mg of standard. If you weighed 19.3 mg of standard, dissolve it in: (19.3mg)/(0.192 mg/mL) = 101 mL of methanol. This makes the high standard solution concentration equal to 0.192 mg/mL.

<u>Low Standard:</u>

The low limit is 85%; therefore the concentration of the low standard = (0.167 mg/mL X 0.85 = 0.142 mg/mL. Dilute 1.00 mL of high standard to 1.35 mL by adding 0.35 mL of methanol (1.15/0.85 = 1.35).

Spotting:

Spot on the 5 X 10 cm silica gel TLC aluminium plate with 3 µL aliquots as follows:

Left spot	low standard (85%) = 0.426 µg
Center Spot	100% sample = 0.501 µg
Right Spot	high standard (115%) = 0.576 µg

Development:

Mix 18.0 mL of ethyl acetate, 4.00 mL acetone, and 0.100 mL glacial acetic acid. Develop the plate in a small glass chamber with approximately 20.0 mL of this solution until the solvent front reaches within 1 cm of the top of the TLC plate.

(R_f = 0.42)

Detection:

<u>UV:</u>

Dry the plate and observe under ultraviolet light at 254 nm. Observe the intensities and the sizes of the spots.

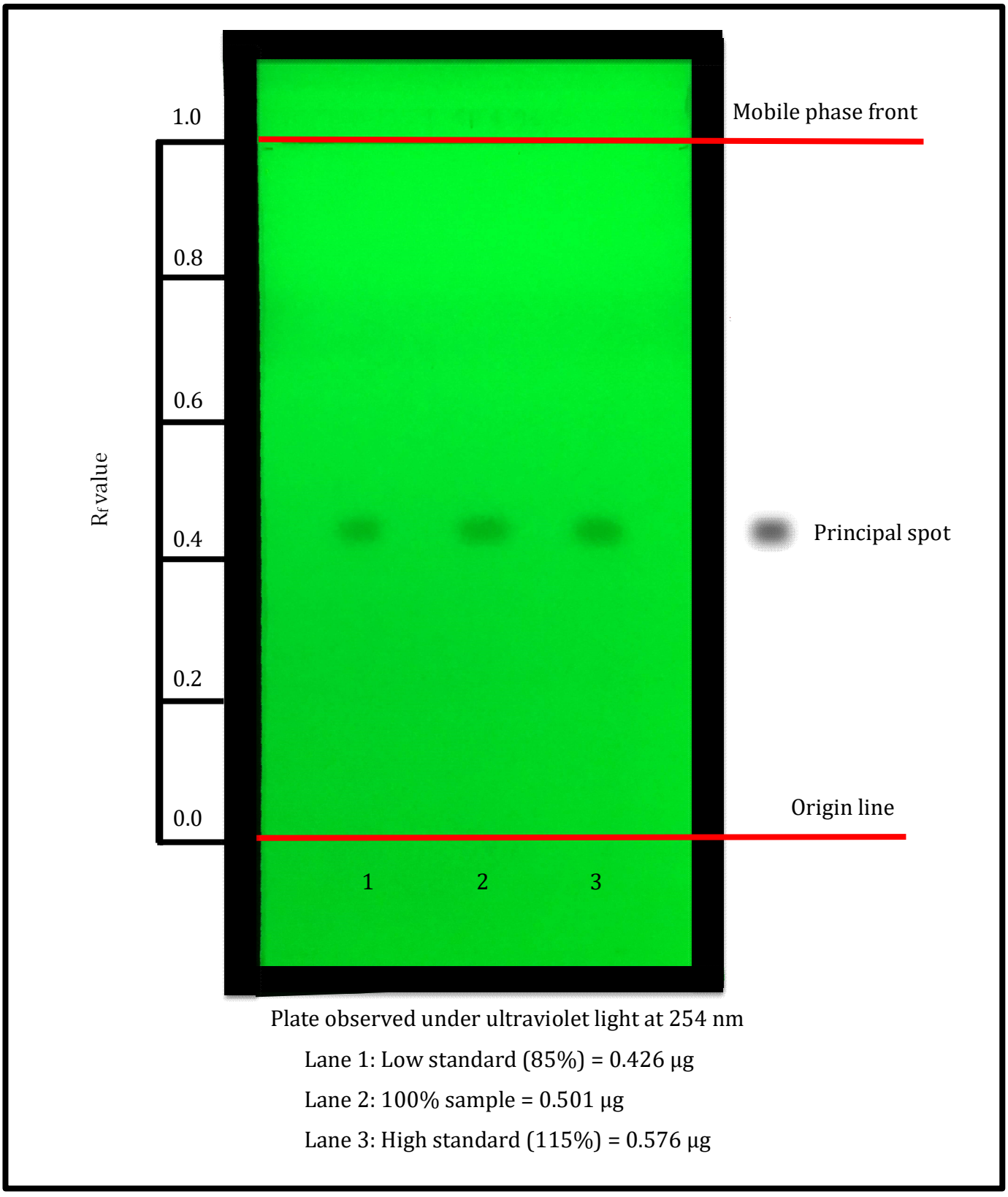


Plate observed under ultraviolet light at 254 nm

Lane 1: Low standard (85%) = 0.426 µg

Lane 2: 100% sample = 0.501 µg

Lane 3: High standard (115%) = 0.576 µg

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