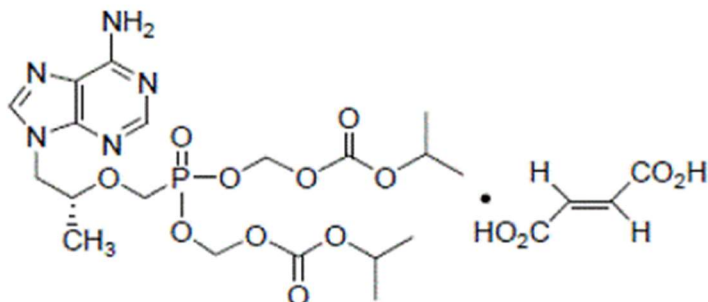


**Tenofovir Disoproxil Fumarate**  
**300 mg Tablet**  
**(in combination with Lamivudine 300mg and Doulegravir 50 mg)**



**Structure:**

**Molecular Formula and Mass:** C<sub>23</sub>H<sub>34</sub>N<sub>5</sub>O<sub>14</sub>P– 635.52 g/mol

**Category:** HIV nucleoside analog reverse transcriptase inhibitor and Anti-HBV reverse transcriptase inhibitor.

**Sample:**

Grind one tablet and dissolve in 100 mL of methanol. Shake for at least 10 min and filter: 300 mg/100 mL = 3.00 mg/mL. Dilute 1.00 mL with an additional 5.00 mL of methanol, for a total volume of 6.00 mL. 3.00 mg/ 6.00 mL = 0.500 mg/mL. Final concentration of sample solutions is 0.500 mg/mL, which is the required concentration representing 100%.

High Standard:

The high limit is 115%; therefore the concentration of the high standard is 0.500 mg/mL × 115% = 0.575 mg/mL. Weigh approximately 57.5 mg of standard and dissolve it in 100 mL of methanol. This makes the high standard solution concentration equal to 1.15 mg/mL, which is 115%.

Low Standard:

The low limit is 85%; therefore the concentration of the low standard = 0.500 mg/mL × 85% = 0.425 mg/mL. Dilute 1.70 mL of high standard to 2.30 mL by adding 0.60 mL of methanol. This gives a concentration of 0.575 mg/mL × 1.70 mL ÷ 2.30 mL = 0.425 mg/mL, which is 85%.

**Spotting:**

Spot on the 5 × 10 cm silica gel TLC aluminum plate with 3.00 µL aliquots as follows:

Left spot	low standard (85%) = 1.28 µg
Center Spot	100% sample = 1.50 µg
Right Spot	high standard (115%) = 1.73 µg

**Development:**

Mix 30.0 mL of ethyl acetate, 7.00 mL of methanol, 3.00 mL of acetone, and 1.00 mL concentrated ammonium hydroxide. 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 = 0.58)

**Detection:**

UV:

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

**Note:**

A screening method for lamivudine is included as Method 6.21 in the Minilab manual, Volume II, pp. 112-115.

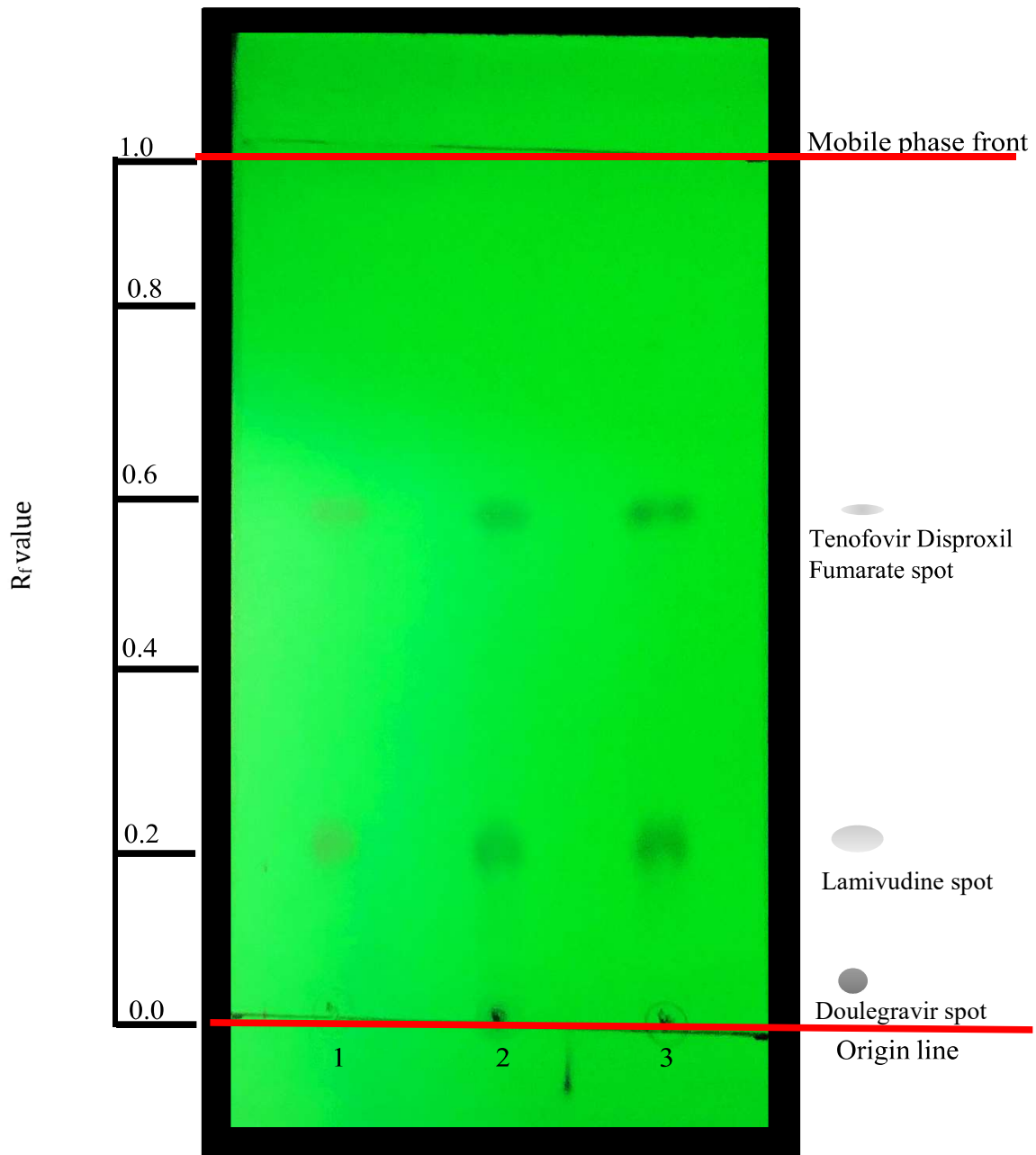


Plate observed under ultraviolet light at 254 nm.

Lane 1: Low standard (85%) = 1.28 µg

Lane 2: 100% sample = 1.50 µg

Lane 3: High standard (115%) = 1.73 µg

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