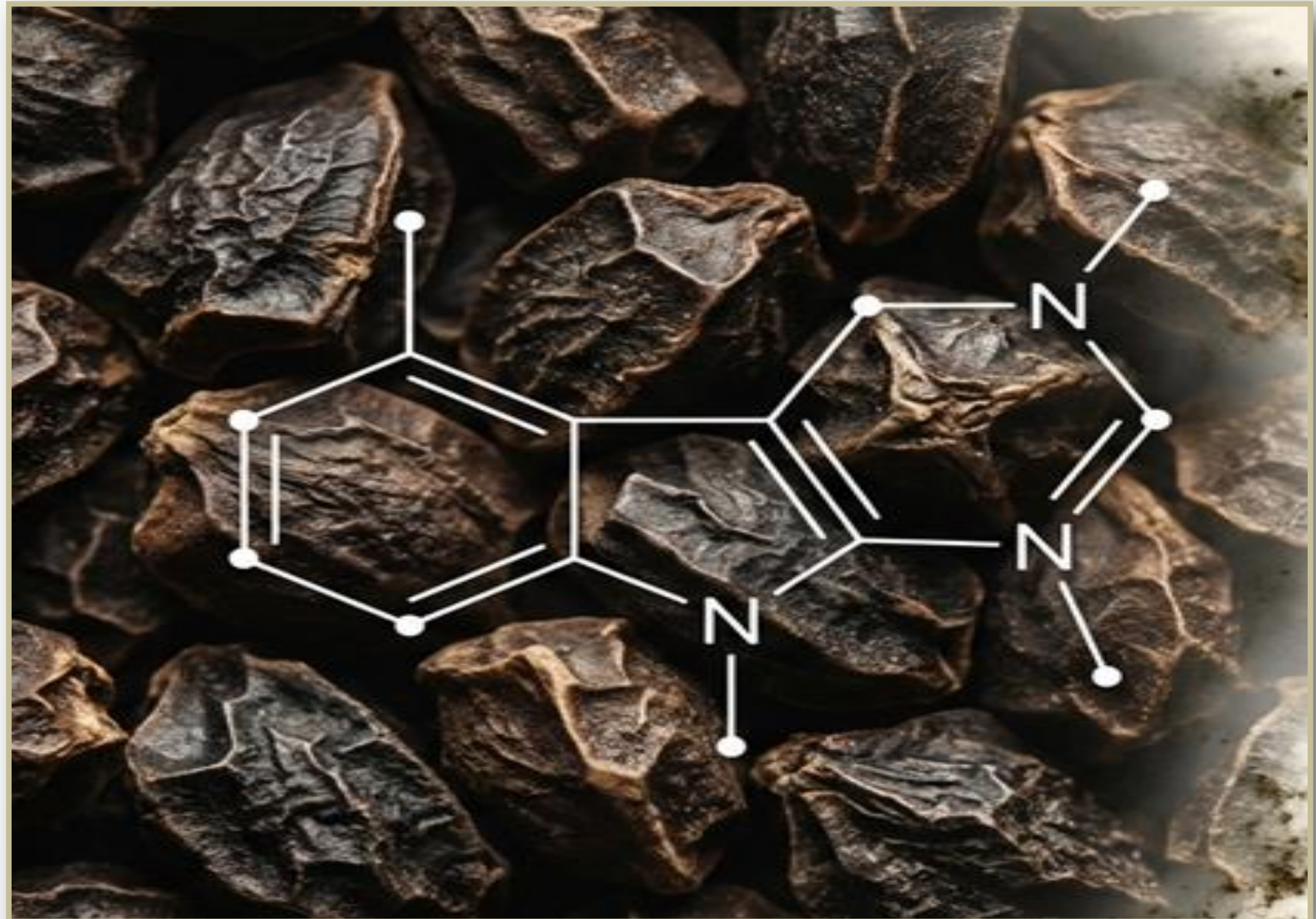


Identification of Harmala Alkaloids

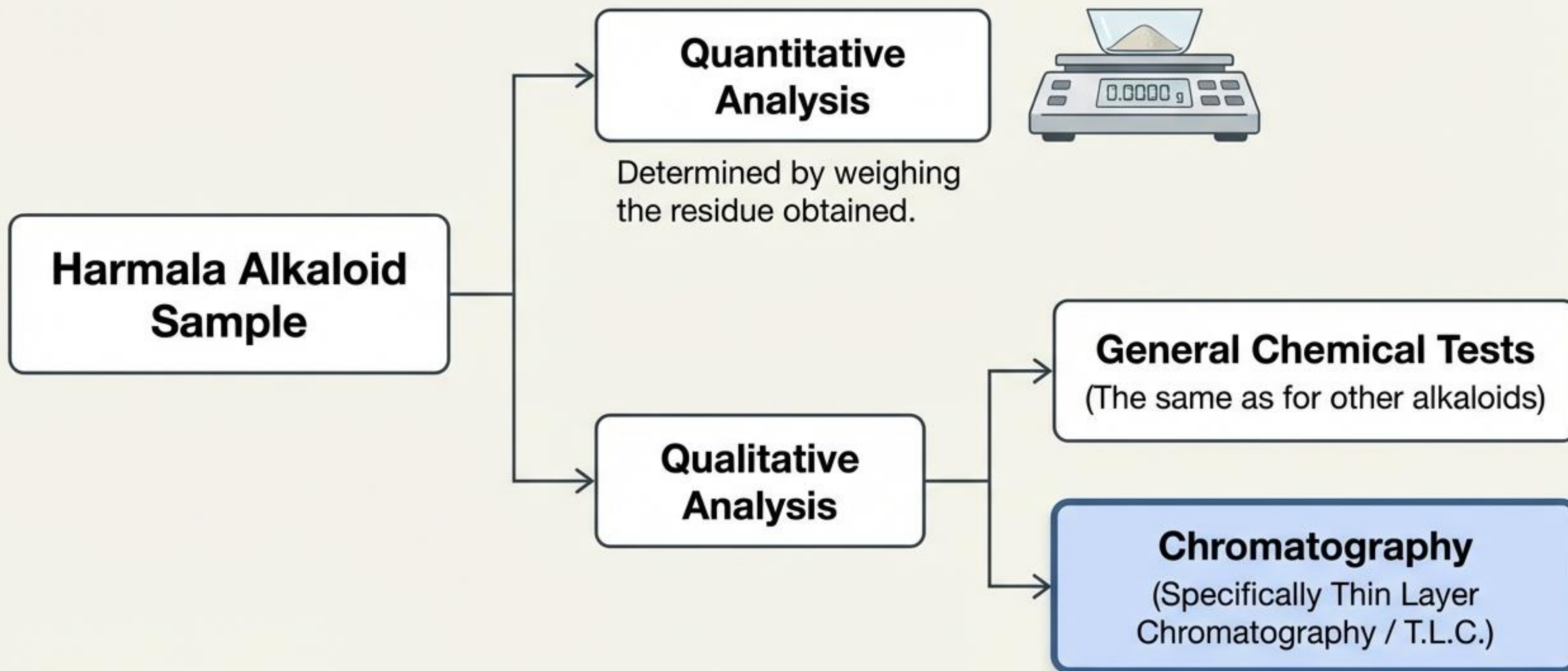
Laboratory Protocol & Analysis (Exp. 2, Part 2)

ASSISST. LEC .
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3RD STAGE
PHARMACOGNOSY DEP. 2026



Pathways for Harmala Alkaloid Analysis



Thin Layer Chromatography (T.L.C.) Parameters

Stationary Phase

Silica gel GF254



Mechanism of Separation

Adsorption



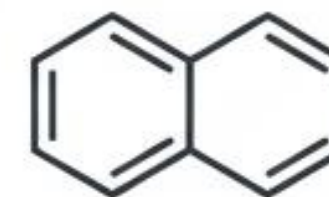
Developing

Ascending



Standard Compound

Any harmala alkaloids



Detection Equipment

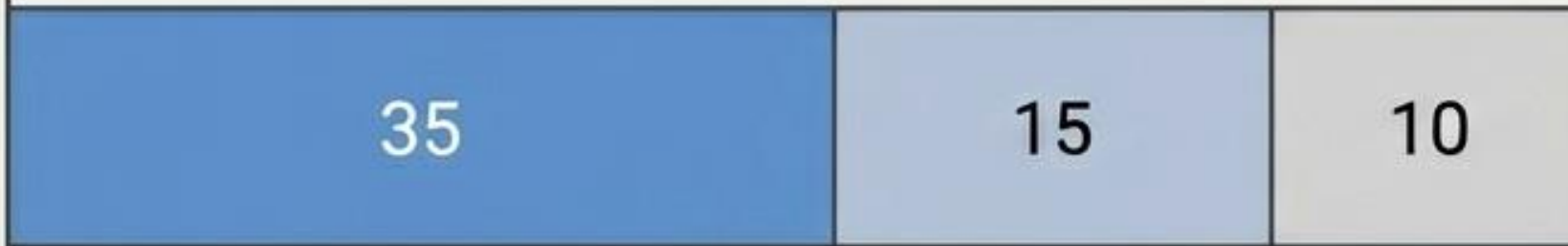
UV Instrument & Spray Reagent
(Dragendorff's reagent)



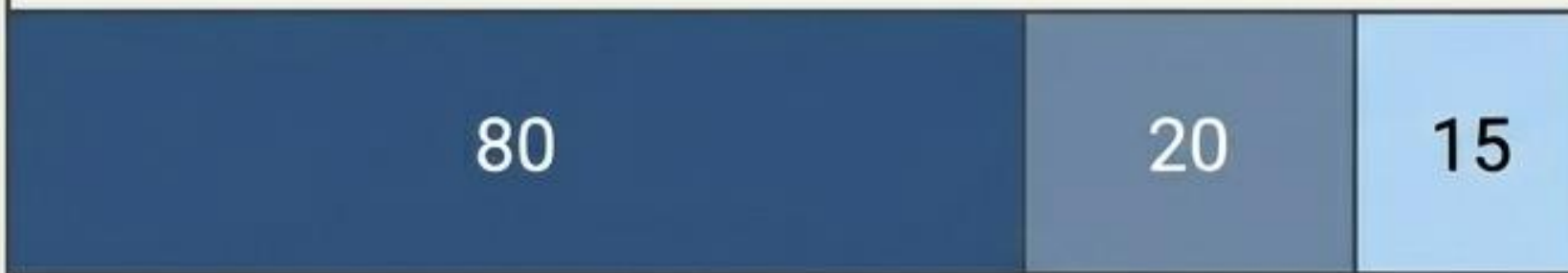
Mobile Phase Formulations

Primary Options

Chloroform : Methanol : Acetone



Chloroform : Methanol : 10% Ammonium hydroxide



Other Mobile Phases

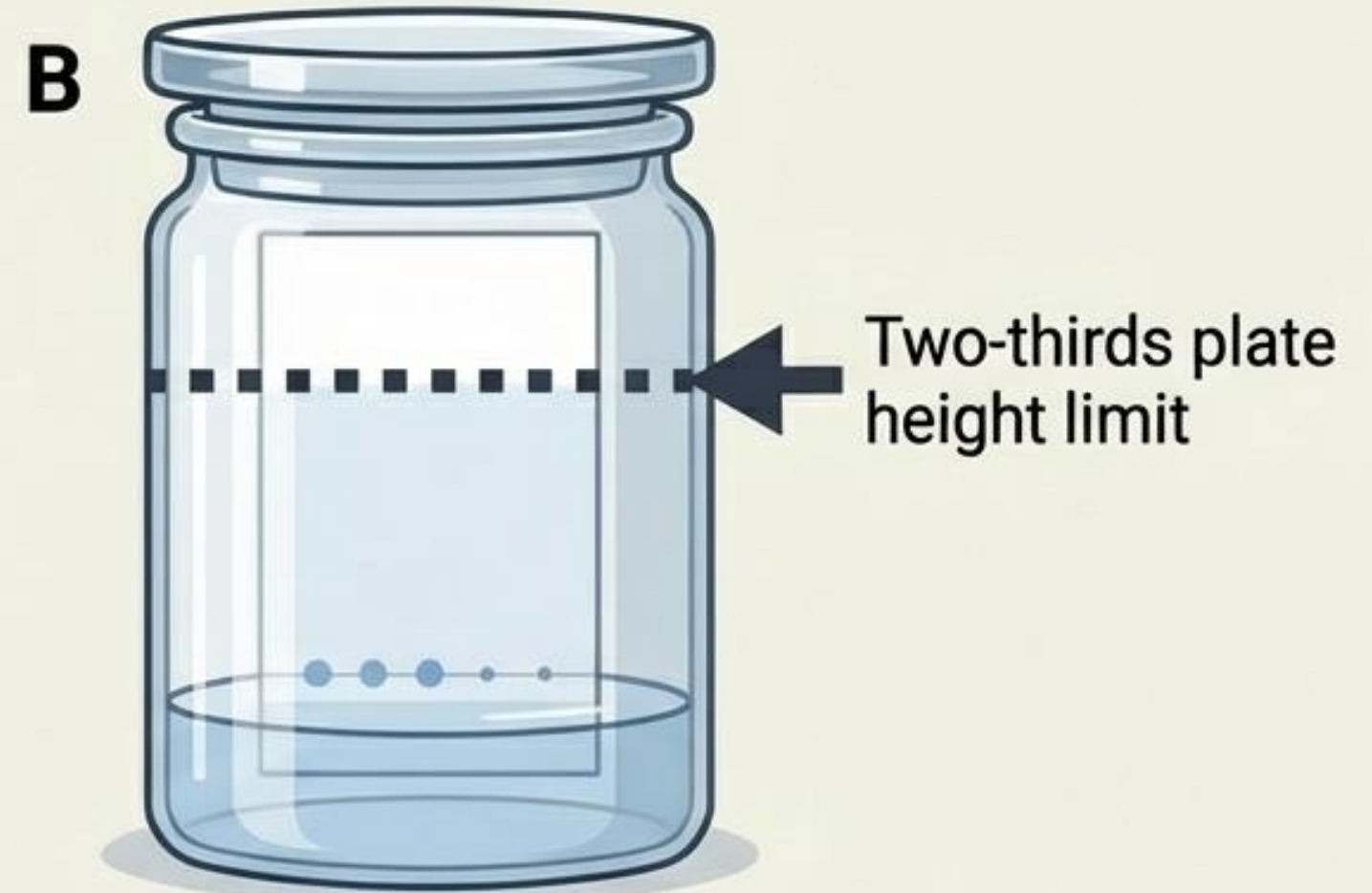
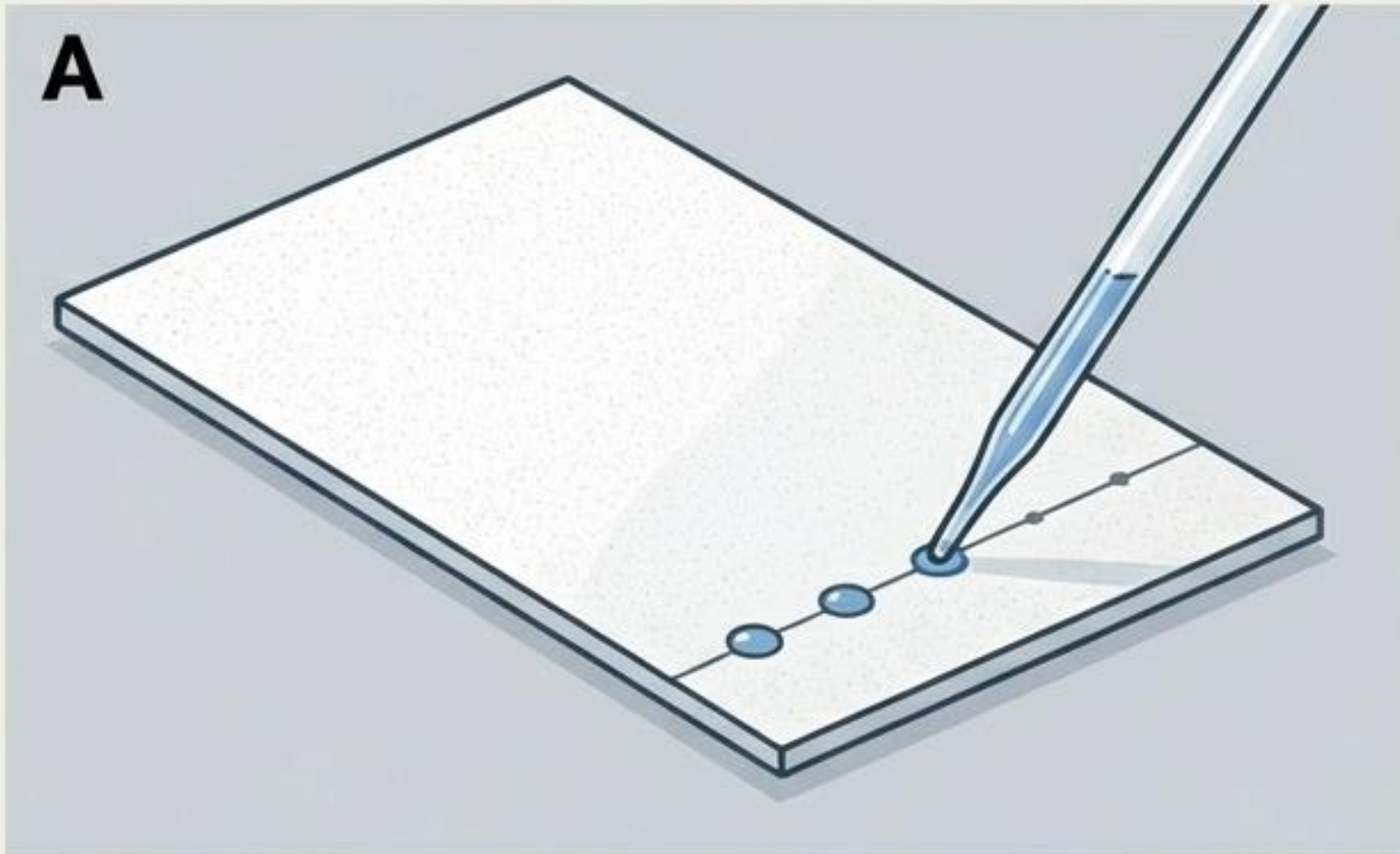
- Chloroform : Acetone : Diethyl amine (50 : 40 : 10)
- Chloroform : Diethyl amine (90 : 10)

Protocol Phase 1: Chamber Preparation & Saturation



1. Prepare the selected mobile phase.
2. Place the mobile phase into the glass jar.
3. Cover the jar with the glass lid.
4. Allow the covered jar to stand for exactly **45 minutes** before use to ensure proper atmospheric saturation.

Protocol Phase 2: Spotting & Developing the Plate

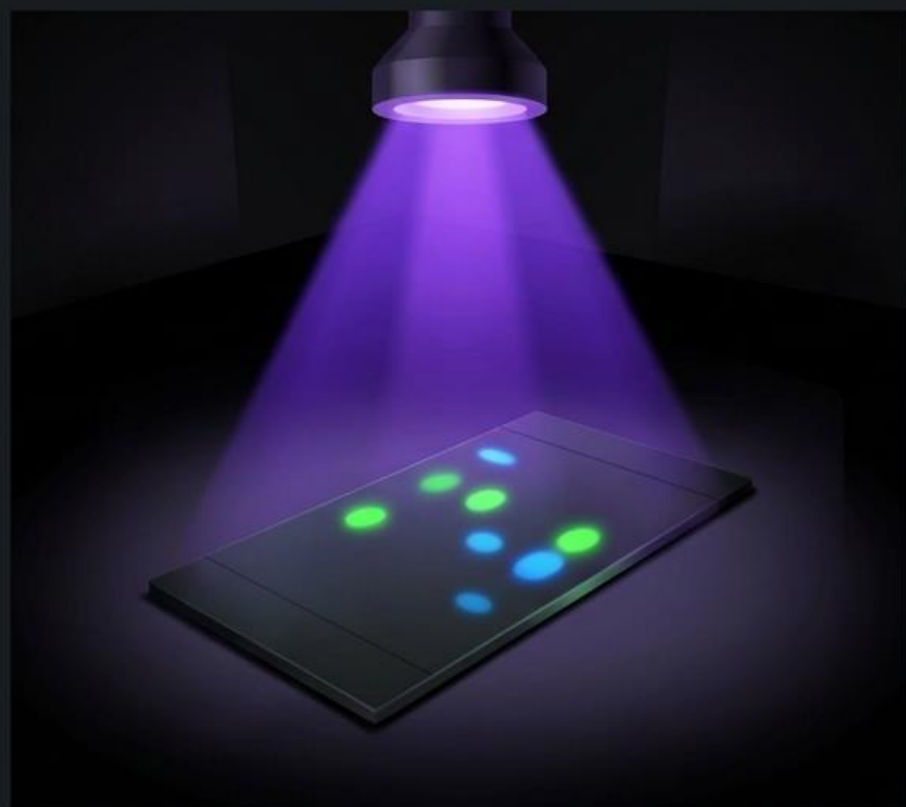


1. Apply the sample and standard spots onto the base line of the silica gel plates using a capillary tube.

2. Put the silica gel plate into the prepared glass jar.

3. Allow the mobile phase to rise to about two-thirds of the plate via ascending development.

Protocol Phase 3: Detection via UV Fluorescence



- 1. Remove the plate from the glass jar and dry completely.
- 2. Identify spots first using a UV instrument.
- 3. Examine the plate under both 254 nm and 366 nm wavelengths.

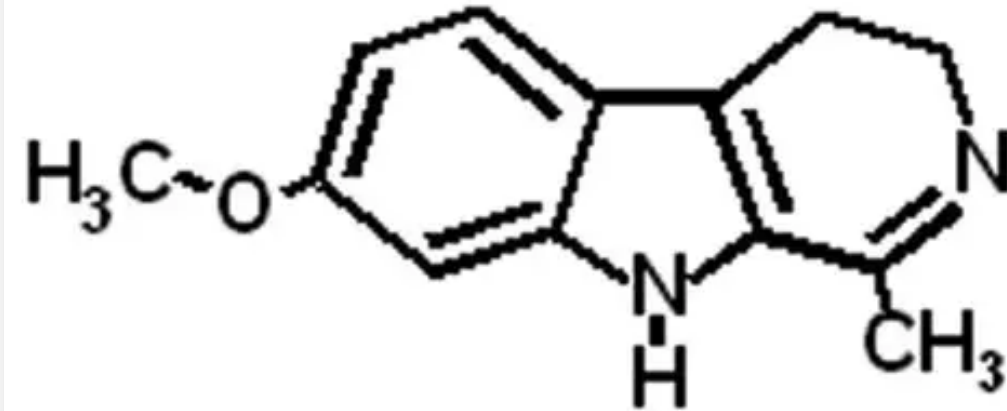
Result: Look for characteristic fluorescence spots appearing under the U.V. light.

Harmala alkaloids are identified by the appearance of **fluorescence spots** when viewed under **UV light at 254 nm and 366 nm**.

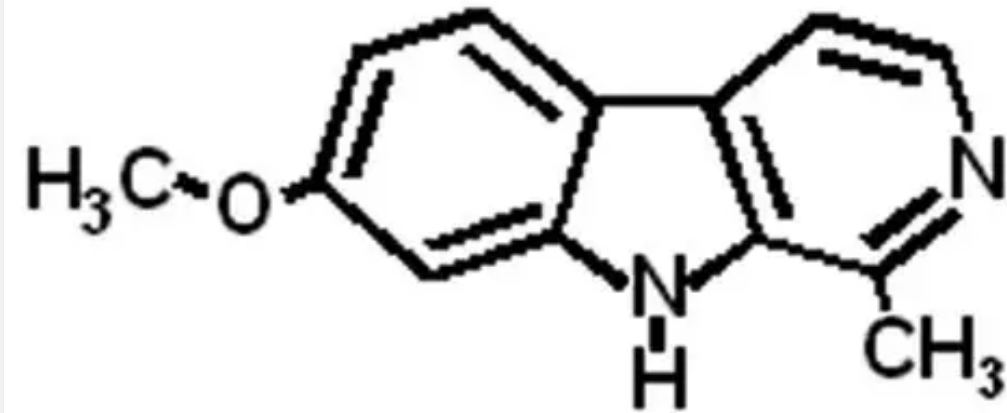
The fluorescence of **harmala alkaloids** (such as **harmine** and **harmaline**) is caused by:

their beta-carboline structure. This chemical structure contains **a highly conjugated system** of double bonds within its aromatic rings. When these molecules are exposed to ultraviolet light, they **absorb the high-energy UV photons**, which excites their electrons to a higher energy state. As the electrons return to their ground state, they release energy in the form of visible light, which is seen as fluorescence.

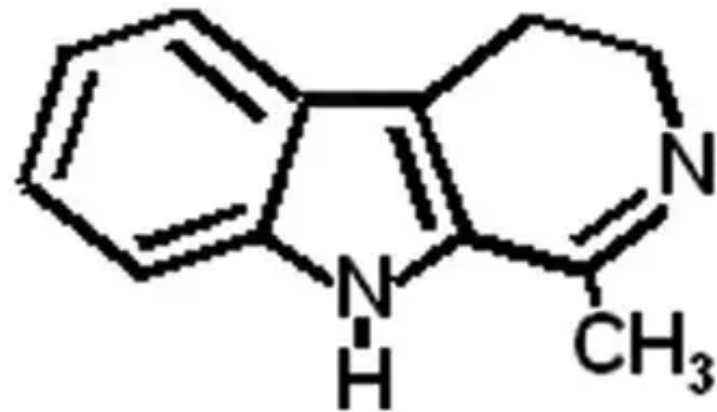
Harmaline



Harminine

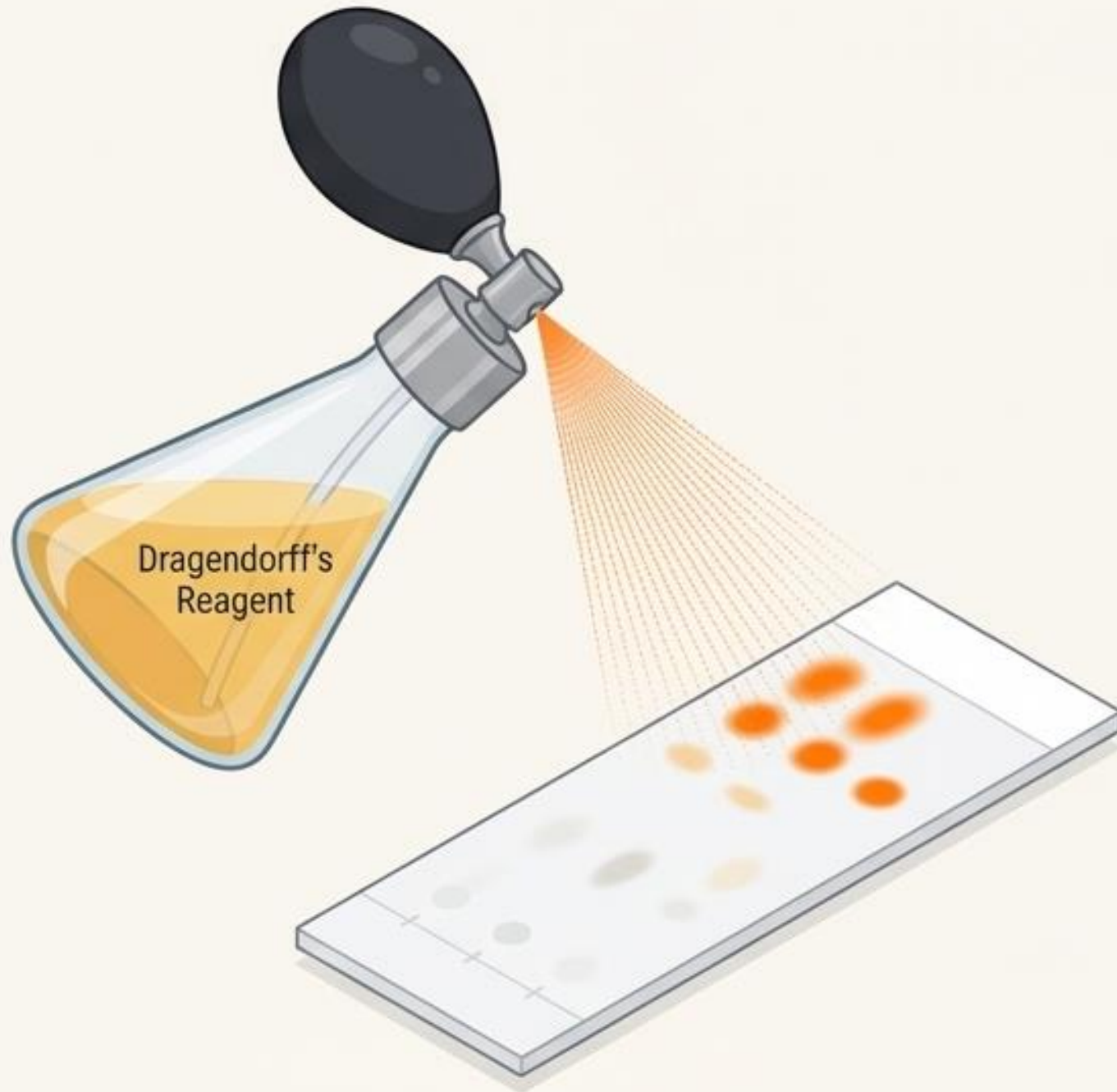


Harmane



اللفظ

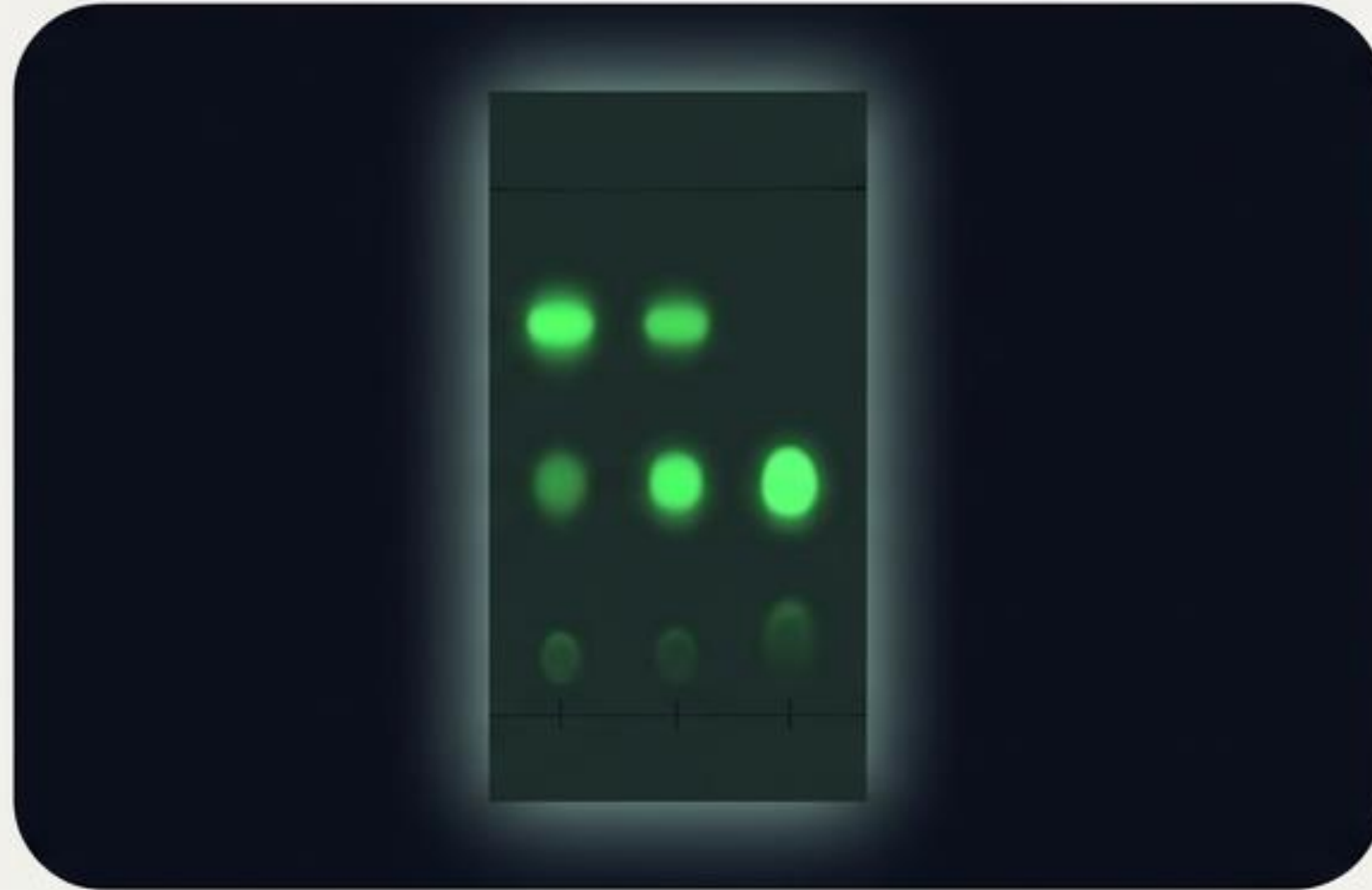
Protocol Phase 4: Chemical Spray Detection



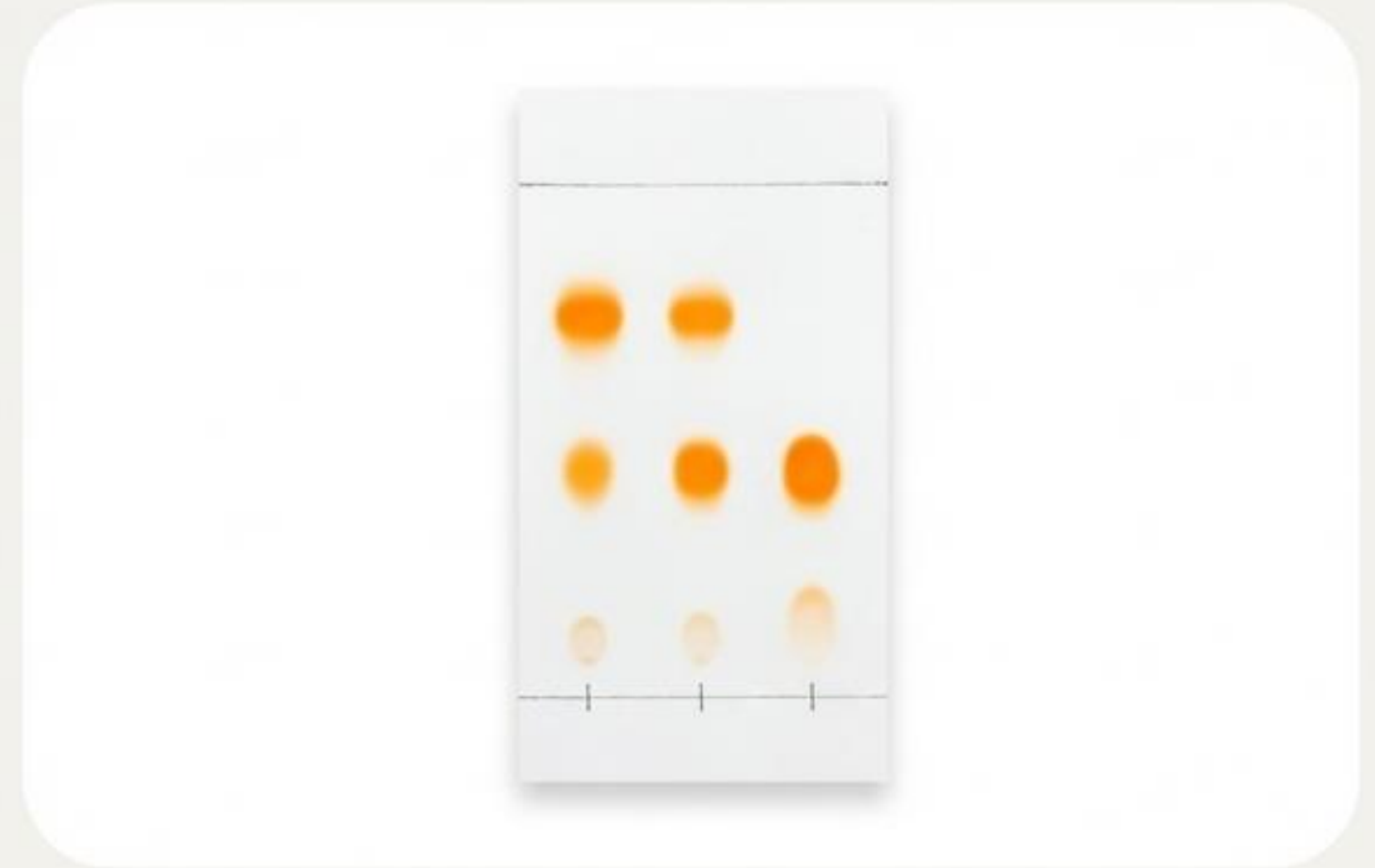
1. Following the UV analysis, proceed to chemical detection.
2. Spray the plate thoroughly with Dragendorff's reagent.

! Result: Look for **definitive** orange spots becoming visible upon reaction with the sprayer.

Final Analysis and Result Interpretation



UV Detection (Fluorescence)



Dragendorff's Reaction (Orange Spots)

Conclusion: By comparing the sample spots to the standard compound (harmala alkaloids), qualitative identification is achieved.

Final Action Required: **Calculate the R_f values** (Retention factor) for the identified spots to finalize the qualitative analysis.

End of Exp. 2 ...Part 2

Thank you
for listening