Practical Pharmacognosy

3rd Stage

1st semester

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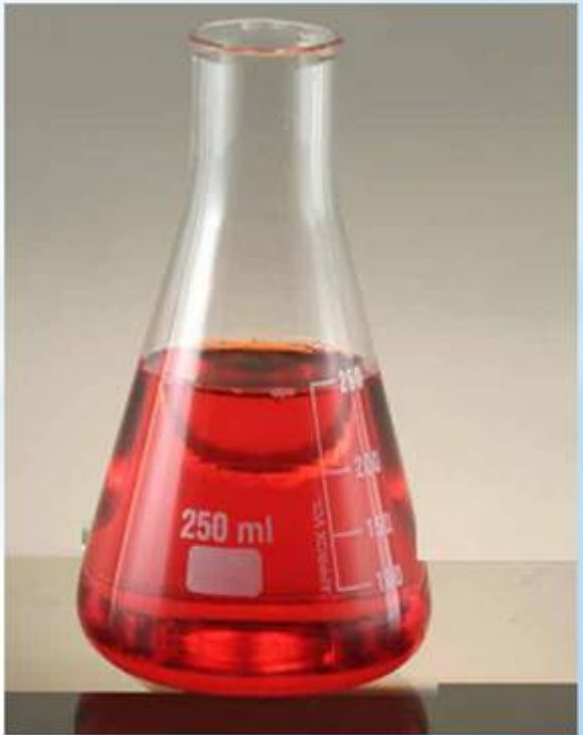
Lab.7

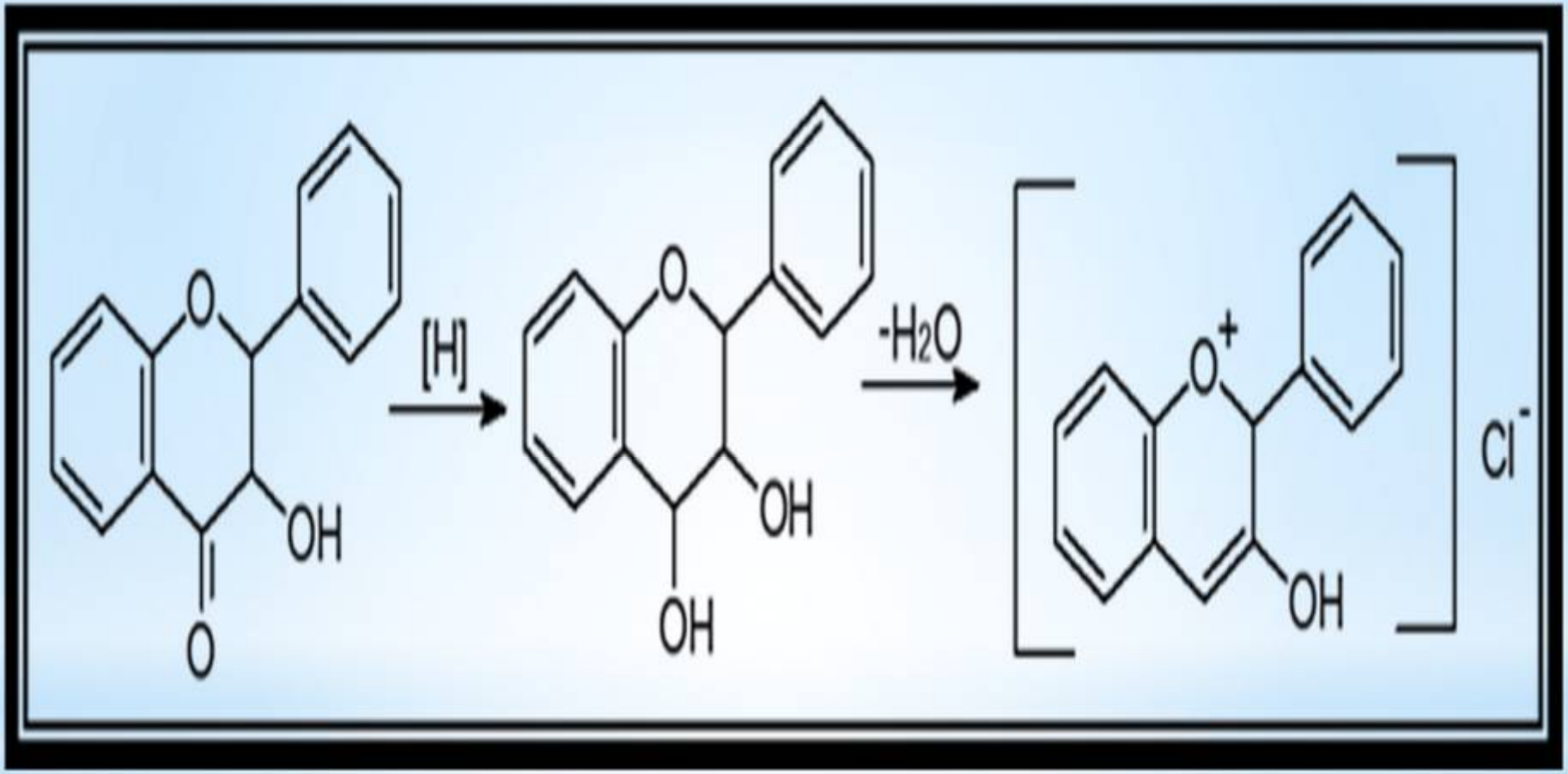
The Chemical Tests

A. General Reaction:

1-Cyanidin reaction with magnesium powder.

Flavanones and dihydroflavanols at the presence of HCl produce bright red colour; isoflavonoids and flavanes develop yellow, sometimes red colour, flavonoles do intensive red colour.




O
OH
O
[H]
O
OH
OH
-H2O
O+
OH
Cl-

2-Reaction of flavonoids with two o-oxygroups in B cycle with lead acetate causes precipitation.

Flavones produce intensive **yellow**, aurones - **red**, anthocyanes **red or blue** precipitates.

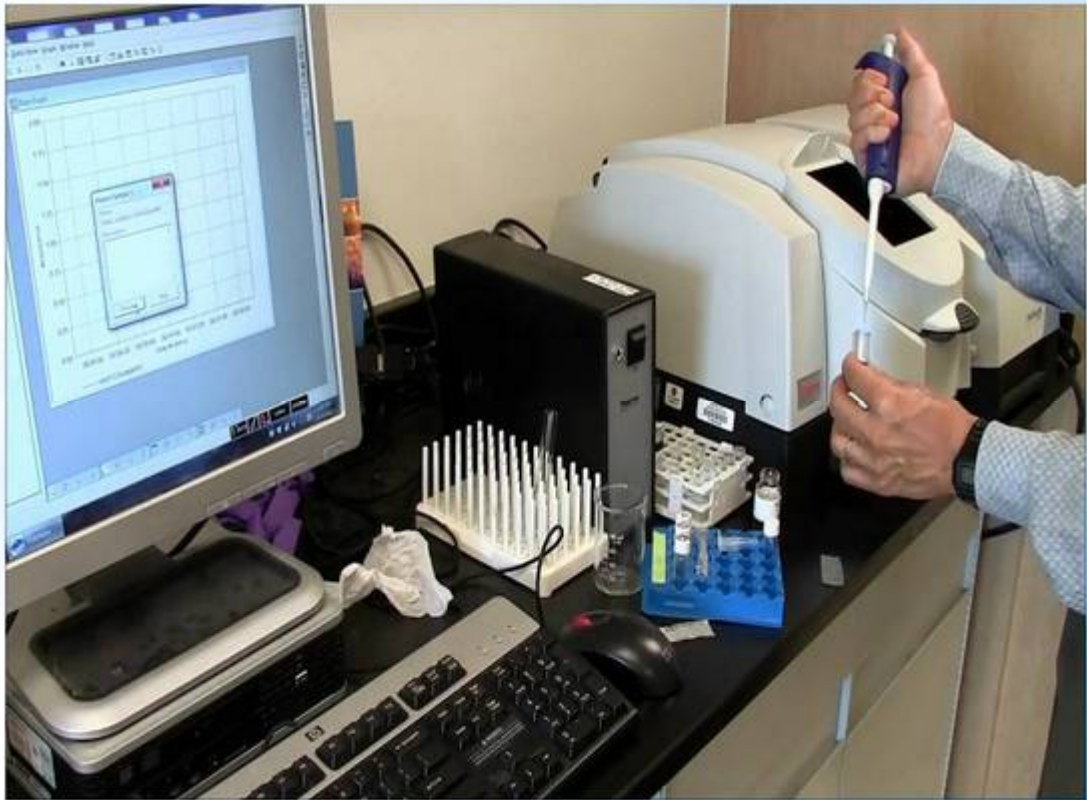
3-Wilson’s reaction: 5-oxyflavones and 5-oxyflavonoles with Wilson's reagent (boric and citric acids in anhydrous acetone) develop brightly **yellow** colour with **yellowish-green** fluorescense.

4-alkaline solution: In alkaline solutions flavanones produce **uncoloured or yellow** precipitates, that for some time become **brightly yellow or yellow** (isomerization to formation of chalkones); chalkones and aurones develop **red** or **purple** (it's their specific reaction), flavones and flavonoles produce **yellow coloured** precipitates.

5-Mineral acids: Flavones and flavonoles with mineral acids form oxonic (flavic) salts of brightly yellow or red colour, chalkones and aurones produce intensive colour of raspberry or red.

6-Other methods: Other methods of identification include chromatography,.colorimetric or spectrophotometric analysis after reaction with aluminium chloride.

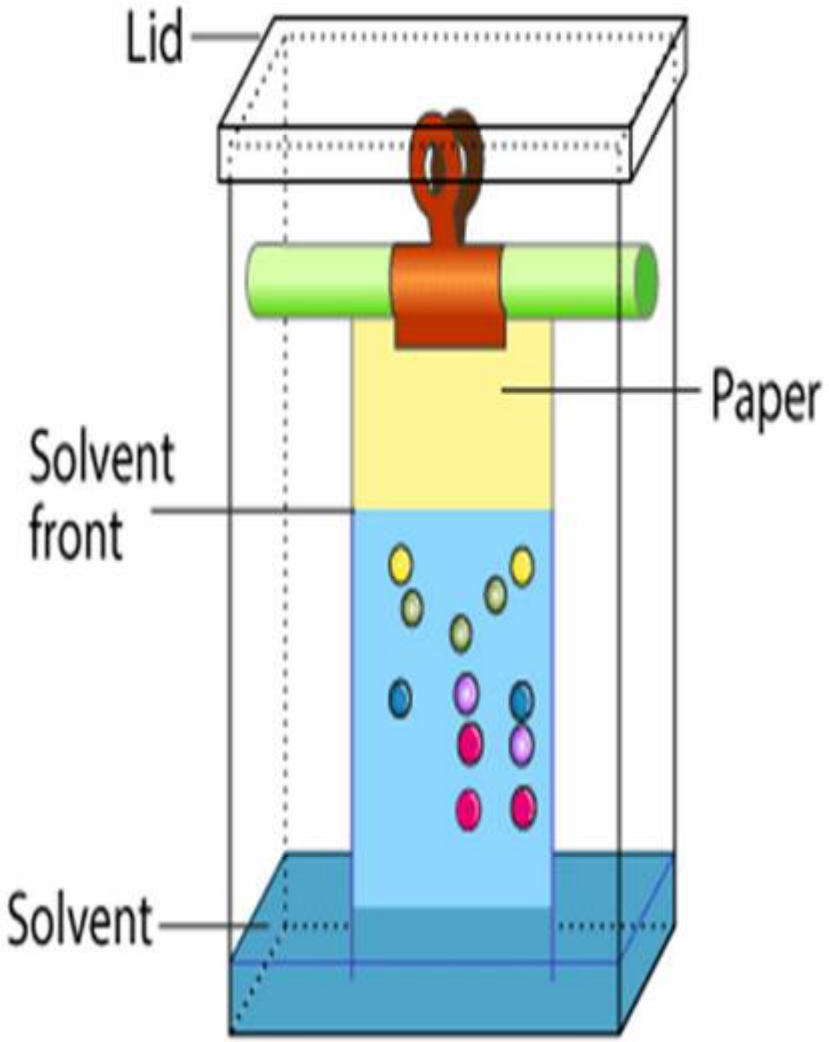
spectrophotometric

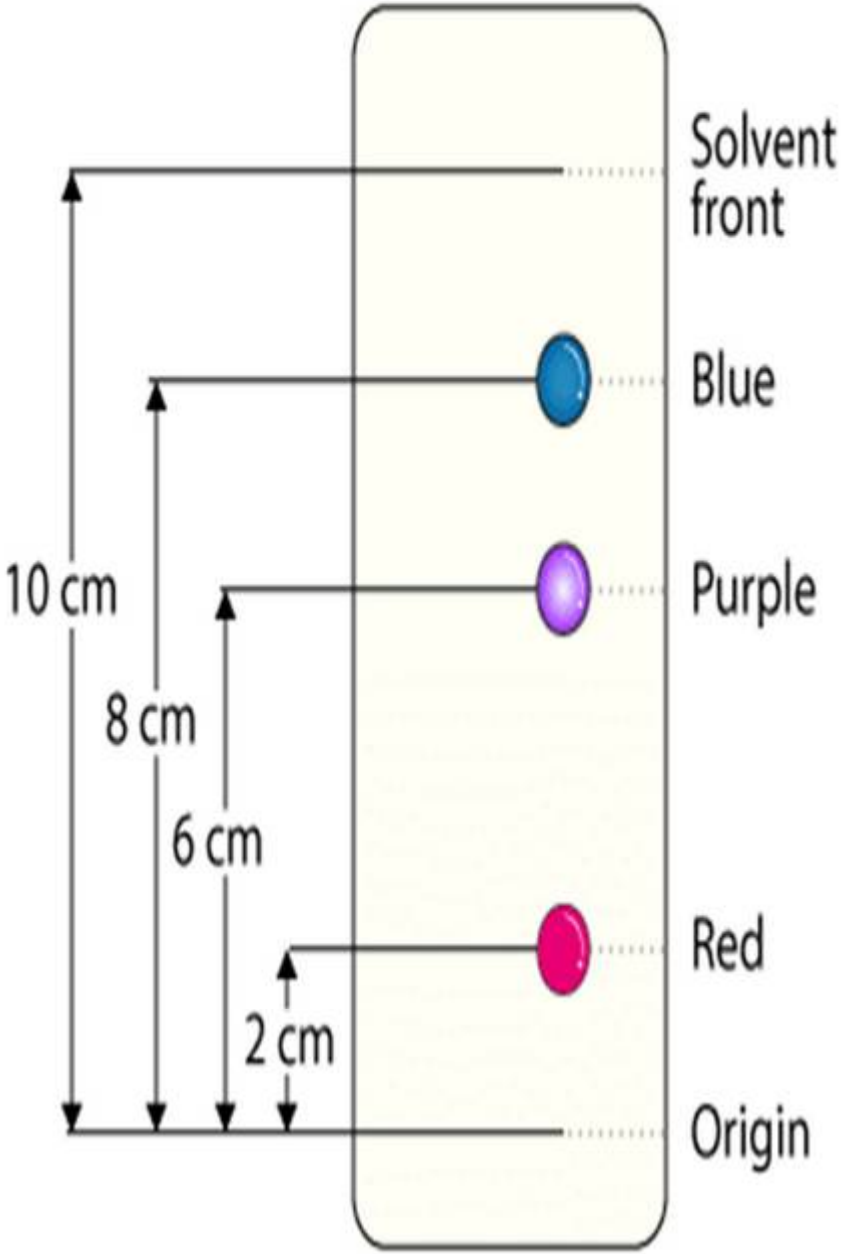


Identification of Flavonoids By Chromatography:

1-By the use of Paper chromatography (P.C):

- The stationary phase = Filter paper (Whatman no.1).
- The mobile phase = n-BuOH:HOAc:H 2O (4:1:5).
- The standard compound = Rutin.
- The spray reagent = 5% alcoholic KOH.
- Mechanism of separation = Partition.
- Developing = Ascending.

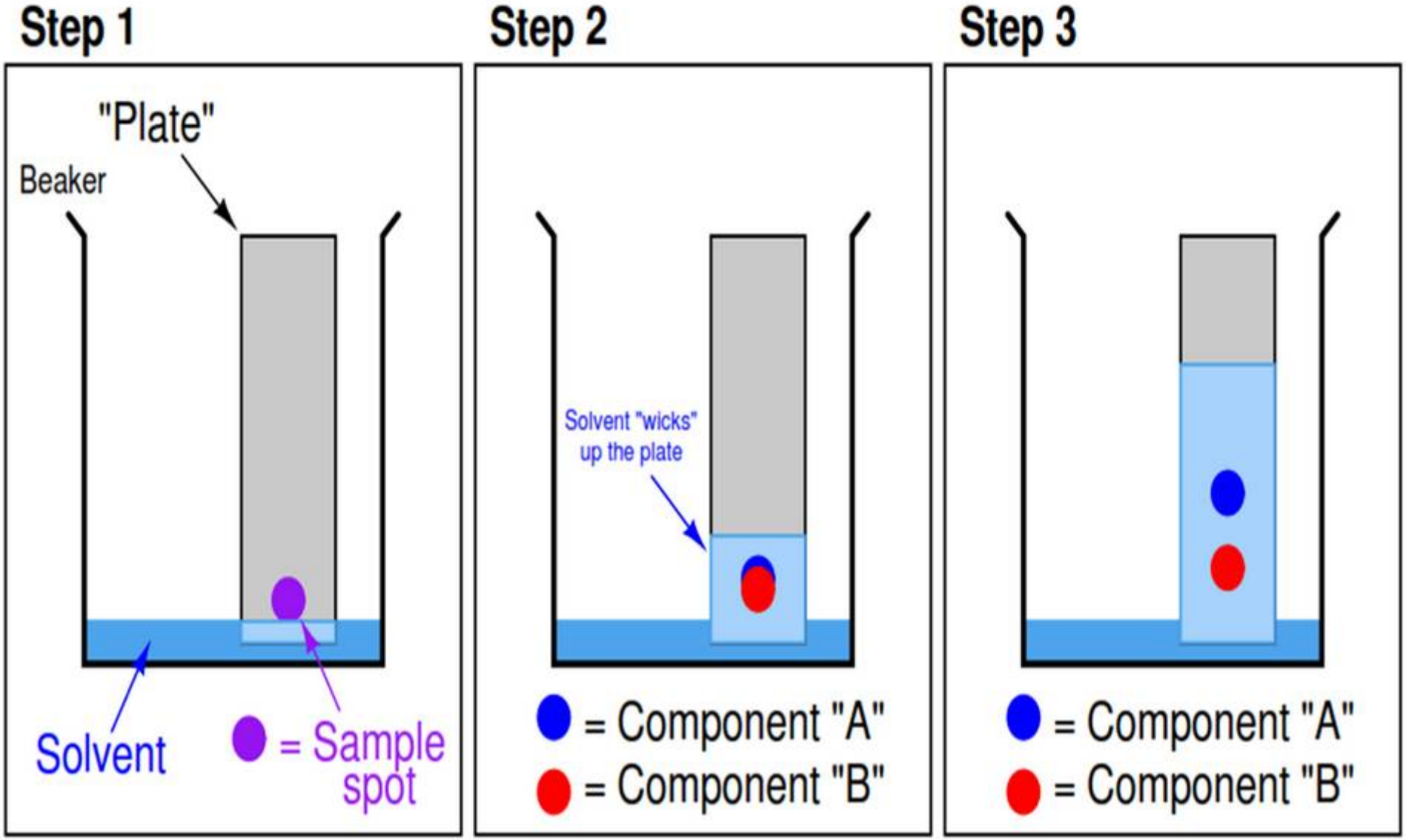

Lid
Paper
Solvent front
Solvent


Solvent front
Blue
Purple
Red
Origin
10 cm
8 cm
6 cm
2 cm

2-By the use of Thin layer chromatography (T.L.C):

- The stationary phase = Silica gel G.
- The mobile phase = Ethyl acetate -formic acid - glacial acetic acid - water(100:11:11:26).
- The standard compound = Rutin.
- The spray reagent = flavonoids spot on TLC plates produce a yellow-brown Spots when reacted with Iodine vapor.
- Mechanism of separation = Adsorption.
- Developing = Ascending.
- Detection: Flavonoids may appear as dark spots on a green background fluoresce when observed in UV light at 254 nm UV-plates containing fluorescent indicator (such as silica gel F254).

Thin-layer chromatography




Thank you