

Aim of the experiment

Systematic extraction, isolation, purification and identification of **neutral poison** (deltamethrin) by various analytical techniques.

Requirements

- a) **Test sample:** Soil sample spiked with 25% EC deltamethrin (commercial).
- b) **Materials:** Beaker(s), test-tubes, test-tube stand, pipette, filter paper sheet, separating funnel, measuring cylinder, glass rod, conical flask, tripod.
- c) **Instrument:** Hot plate, UV light chamber.
- d) **Chemicals:** Sulfuric acid, sodium hydroxide, iodine solution, hexane, ferrous sulphate, silver nitrate, iodine, benzene.

Theory and Principle

- a. Pyrethroids are synthetic analogues. Toxicity is very low due to their rapid metabolism. e.g. deltamethrin, d-allethrin, permethrin. They are used as insect repellent, and pesticides commercially available as sprays, dust, powders, mats, and coils. Deltamethrin is a neurotoxin, it temporarily attacks the nervous system. Skin contact can lead to tingling or reddening of the skin local to the application. If taken through the eyes or mouth, common symptoms are facial paraesthesia, with a feel of abnormal sensation including burning, partial numbness, pins and needles, skin crawling etc.
- b. Thin layer chromatography, or TLC, is a method for analysing mixtures by separating the compounds in the mixture. After the sample has been applied on the plate, a solvent or solvent mixture (known as the mobile phase) is drawn up the plate via capillary action. Because different analytes ascend the TLC plate at different rates owing to affinity with stationary phase, separation is achieved. The R_f value is used to quantify the movement of the materials along the plate. R_f is equal to the distance travelled by the substance divided by the distance travelled by the solvent. Its value is always between zero and one. The R_f for a compound is a constant from one experiment to the next only if the chromatography conditions such as solvent system; adsorbent; thickness of the adsorbent; amount of material spotted and temperature are also constant.
- c. Beer-Lambert's law: According to this law, when a monochromatic light passes through a transparent solution, the rate of decrease in the intensity of transmitted light w.r.t. thickness and concentration of solution is directly proportional to the frequency of incident light.
$$A = \log I_0/I_t = \epsilon cl$$

Where, c = concentration of solution; l = thickness of solution, E = constant; A = Absorbance

Procedure

1. Extraction of deltamethrin from soil sample:

- a) Weigh about 20g of soil sample in a beaker and transfer to separating funnel.
- b) 60 ml of hexane is added in a separating funnel and shook to-fro for 1 hour.
- c) The separating funnel is then kept then stand on tripod for five-ten minutes and then filtered.
- d) Filtrate is passed through the activated charcoal, to de-colorize and further can be used for analysis.
- e) Filtrate is evaporated on hot plate to obtain a dry residue which was further reconstituted in methanol for analysis.

2. Reagent Preparation

Sodium hydroxide solution (10%): 10 gram of sodium hydroxide was added in volumetric flask and made up to 100 ml with distilled water.

Ferric sulfate solution (5%): 5 gram of ferric sulfate was added in volumetric flask and made up to 100 ml with distilled water.

Iodine solution (1%): 1 gram of iodine was added in volumetric flask and made up to 100 ml with benzene.

3. Colour test:

Test	Procedure	Observation	Inference
1	Extract + 10% sodium hydroxide solution + 5% freshly prepared aq. ferrous sulphate heat + dilute sulphuric acid	Crucian blue colour will appear.	Indicates presence of Deltamethrin
2	Extract + 1% ammonium silver nitrate exposed to UV light.	Black colour will be observed	
3	Extract +1% iodine solution in benzene	Yellowish brown colour will be observed.	

4. Thin layer Chromatography: -

Stationary Phase: Silica Gel-G

Mobile phase: Hexane: Acetone (8:2); Hexane: Acetone (1:1)

Preparation and Activation of TLC plates

- a. The glass TLC plates should be cleaned with water and then air-dried.
- b. The silica gel slurry is prepared by mixing a sufficient amount of silica gel-G with distilled water in the ratio of 2:1 while stirring it slowly with a glass rod, till the desired consistency (with homogenous nature) is achieved.
- c. Pour the slurry onto the glass slide from one side and spread the slurry evenly to obtain uniform thickness. Let the plates air dry.
- d. Before an hour or two of experiment TLC plates keep the plates for activation by placing in a thermostatically controlled oven at 110°C for a duration of 60 minutes.
- e. TLC plate is observed for non-uniformity or if any cracks develop owing to over activation, such plates must be discarded.

Preparation of mobile phase

In TLC tank / TLC development chamber, 100 ml solvent system to be prepared by mixing acetone and hexane in 8:2 and 1:1 .

Chamber should be covered with a lid and leave to stand for at-least 30 minutes for the saturation of chamber such that equilibrium is obtained before placing the TLC plate for development.

Spotting of sample

- a. Spotting is done using a capillary tube immersed into the sample solution such that liquid is drawn into the capillary.
- b. Very gently the end of the capillary tube to be pressed on the prepared TLC plate at a distance of 2 cm from the base. Carefully spot the sample to avoid tailing of sample as well as contaminating the adjacent sample. The spot to be air-dry and repeated spotting can be done directly over the original spot, if needed.
- c. Two spots to be done; one the extracted sample and other, standard in methanol.

TLC plate development

- a. Before placing the TLC plate in the developing chamber, double check the plates, such that the solvent level is below the sample spot. The sides of the plate did not touch the jar.
- b. Place the plate vertically in the developing jar with the top end leaning against the jar.
- c. The jar is sealed and allow the solvent to run to rise until 2-3 cm from the top of the plate.

- d. Take out the plate from the tank and mark immediately for the solvent front with a pencil. Allow the plate to dry.

Visualization of sample

The plate is sprayed with phenylhydrazine to view the spots.

5. UV-Visible spectroscopy: -

- a) Instrument is switched on and allow it to stand for 20 min for warm-up/ check parameters.
- b) Cuvettes should be rinsed with methanol and the baseline run is produced using methanol in both reference and sample cell covered with lid.
- c) The extracted sample and standard reconstituted in methanol are analyzed in spectra mode.
- d) Cuvettes were rinsed using methanol, further with distilled water and placed in case. Shut down the instrument to printing of spectra.
- e)

Observation and Calculation

1. Thin Layer Chromatography

Rf value of Deltamethrin (extracted sample):

Distance travelled by solute (x) = ___ cm

Distance travelled by solvent (y)= ____ cm

Rf value = x / y =

Rf value of Deltamethrin (commercial standard):

Distance travelled by solute = ___ cm

Distance travelled by solvent = ____ cm

Rf value = x / y =

2. UV-Visible spectroscopy

The spectra indicate peaks at _____ nm, which are prominent to deltamethrin.

Conclusion: On the basis of observations from color test, TLC and UV analysis, it can be concluded that the test sample was deltamethrin, a pyrethroid insecticide.